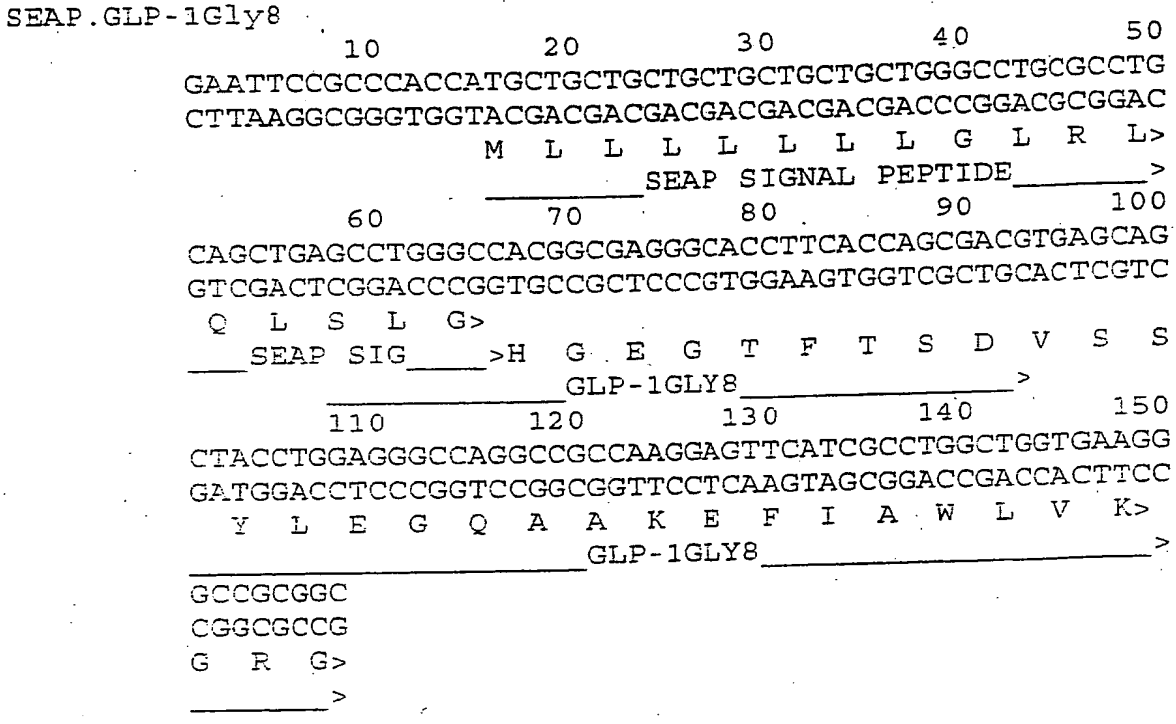

SEAP.GLP-1Gly8
10 20 30 40 50
GAATTCCGCCCACCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGCGCCTG
CTTAAGGCGGGTGGTACGACGACGACGACGACGACGACCCGGACGCGGAC
M L L L L L L L G L R L>
SEAP SIGNAL PEPTIDE>
60 70 80 90 100
CAGCTGAGCCTGGGCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAG
GTCGACTCGGACCCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTC
Q L S L G>
SEAP SIG >H G E G T F T S D V S S
GLP-1GLY8>
110 120 130 140 150
CTACCTGGAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGG
GATGGACCTCCCGGTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCC
Y L E G Q A A K E F I A W L V K>
GLP-1GLY8>
GCCGCGGC
CGGCGCCG
G R G>
>

Figure 1

Exendin-4.GLP-1Gly8

```
          10         20         30         40         50
GAATTCCGCCCACCATGAAGATCATCCTGTGGCTGTGTGTGTTCGGCCTG
CTTAAGGCGGGTGGTACTTCTAGTAGGACACCGACACACACAAGCCGGAC
                M  K  I  I  L  W  L  C  V  F  G  L>
               _____________PROEXENDIN_____________>

          60         70         80         90         100
TTCCTGGCCACCCTGTTCCCCATCAGCTGGCAGATGCCCGTGGAGTCCGG
AAGGACCGGTGGGACAAGGGGTAGTCGACCGTCTACGGGCACCTCAGGCC
 F  L  A  T  L  F  P  I  S  W  Q  M  P  V  E  S  G>
_____________________PROEXENDIN_____________________>

         110        120        130        140        150
CCTGTCCTCCGAGGACTCCGCCAGCTCCGAGAGCTTCGCCAAGCGCATCA
GGACAGGAGGCTCCTGAGGCGGTCGAGGCTCTCGAAGCGGTTCGCGTAGT
  L  S  S  E  D  S  A  S  S  E  S  F  A  K  R  I>
_____________________PROEXENDIN_____________________>

         160        170        180        190        200
AGCGCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTGGAG
TCGCGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGACCTC
       H  G  E  G  T  F  T  S  D  V  S  S  Y  L  E>
     _________________GLP-1 GLY-8_________________>
K  R>
_____>

         210        220        230        240        250
GGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGCTG
CCGGTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCGAC
 G  Q  A  A  K  E  F  I  A  W  L  V  K  G  R  G>
_____________GLP-1 GLY-8_____________________>
```

Figure 2

Helodermin.GLP-1Gly8

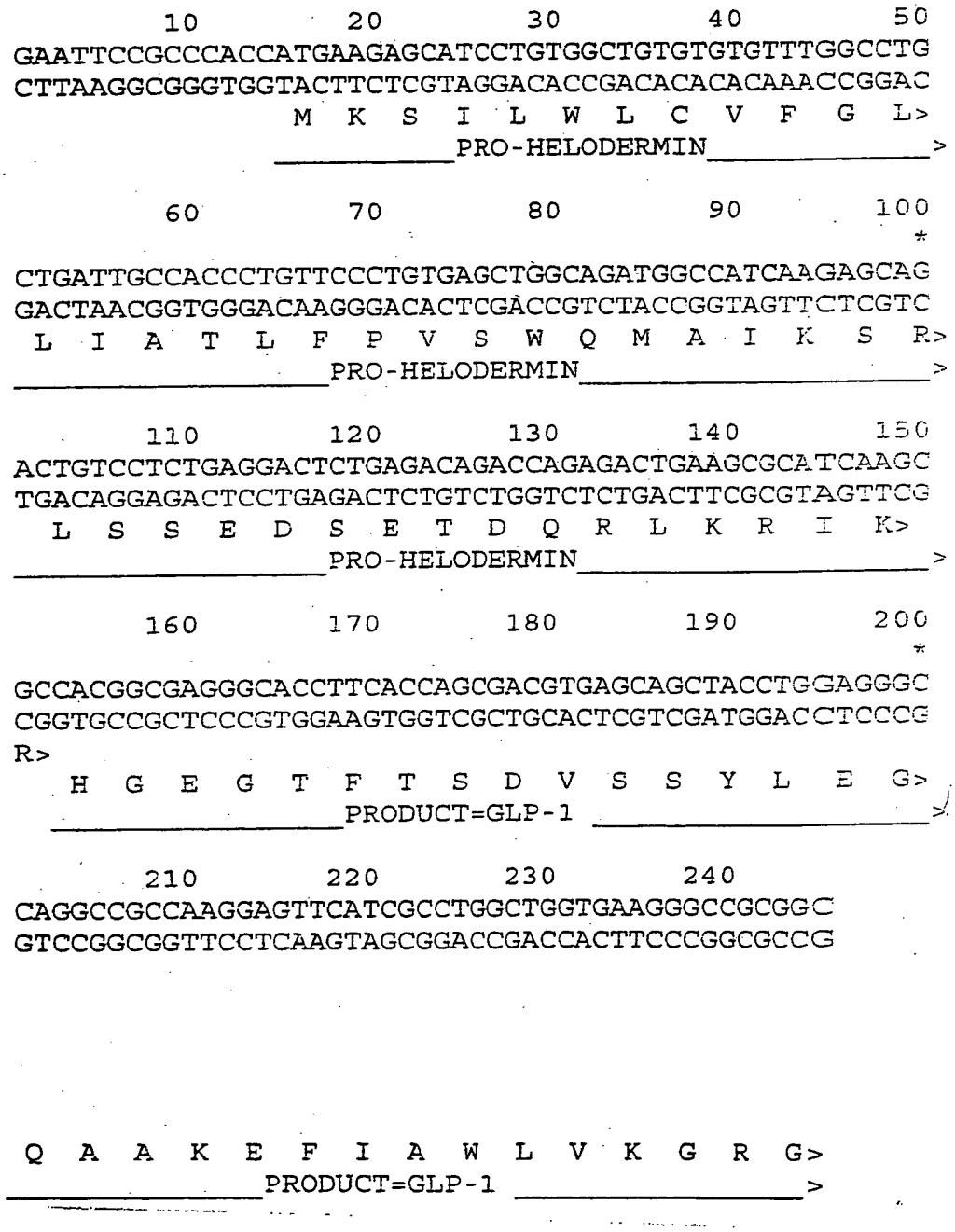


Figure 3

GIP.GLP-1Gly8

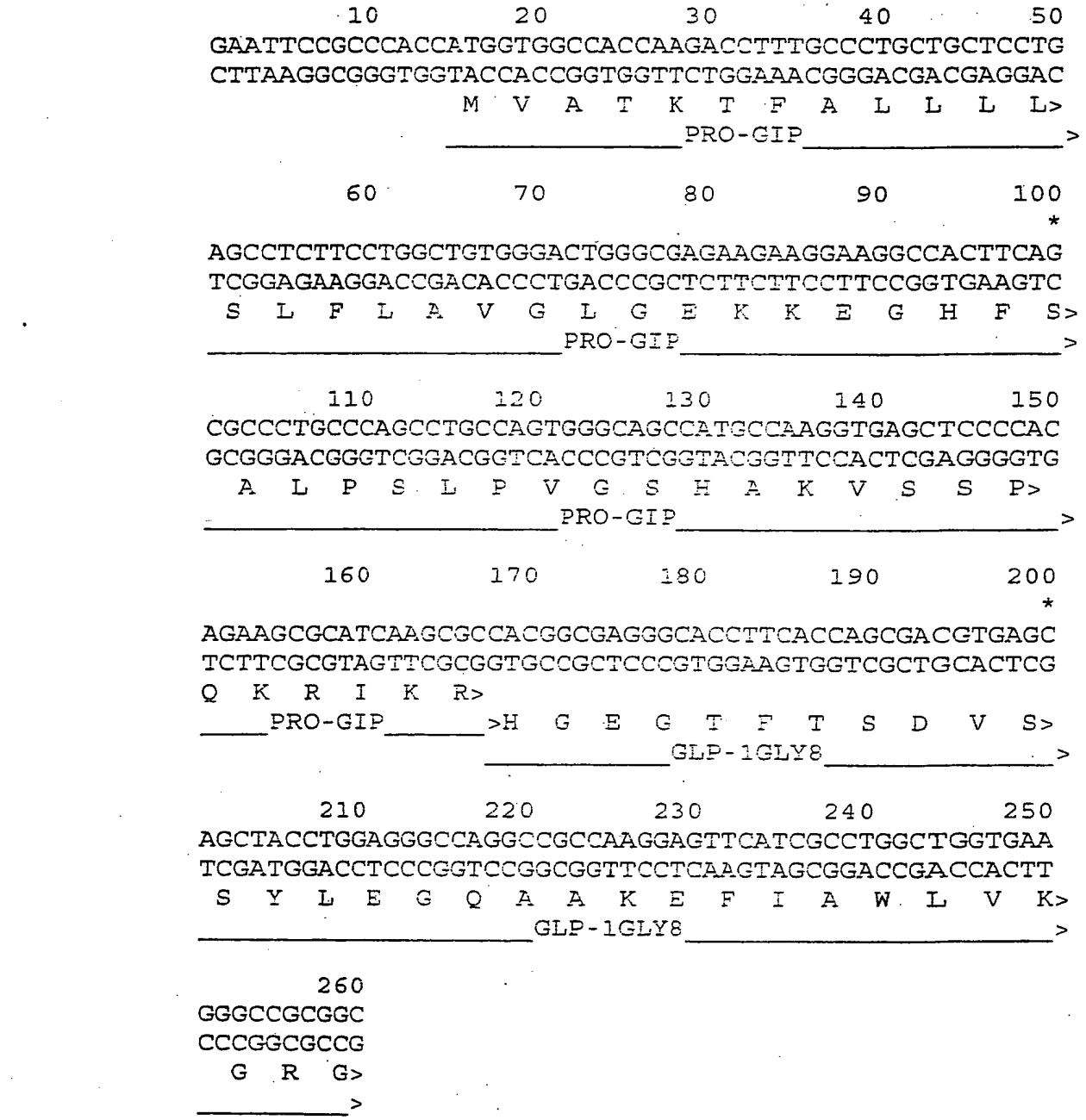


Figure 4

IGF-I(furin).GLP-1Gly8

```
                  10         20         30         40         50
GAATTCCGCCCACCATGGGCAAGATCAGCAGCCTGCCCACCCAGCTGTTC
CTTAAGGCGGGTGGTACCCGTTCTAGTCGTCGGACGGGTGGGTCGACAAG

                    M  G  K  I  S  S  L  P  T  Q  L  F>
                   _____________IGF1 1-48_____________>

          60         70         80         90        100
                                                       *
AAGTGCTGCTTTTGTGACTTCCTGAAGGTGAAGATGCACACCATGAGCTC
TTCACGACGAAAACACTGAAGGACTTCCACTTCTACGTGTGGTACTCGAG
 K  C  C  F  C  D  F  L  K  V  K  M  H  T  M  S  S>
_____________________IGF1 1-48_____________________>

        110        120        130        140        150
CAGCCACCTGTTCTACCTGGCCCTGTGCCTGCTGACCTTCACCAGCTCCG
GTCGGTGGACAAGATGGACCGGGACACGGACGACTGGAAGTGGTCGAGGC
  S  H  L  F  Y  L  A  L  C  L  L  T  F  T  S  S>
_____________________IGF1 1-48_____________________>

        160        170        180        190        200
                                                       *
CCACAGCCAAGCGCATCAAGCGCCACGGCGAGGGCACCTTCACCAGCGAC
GGTGTCGGTTCGCGTAGTTCGCGGTGCCGCTCCCGTGGAAGTGGTCGCTG
A  T  A>
_________>K  R  I  K  R>
         ___FURIN CL____>H  G  E  G  T  F  T  S  D>
                        ______PRODUCT=GLP-1 _________>

        210        220        230        240        250
GTGAGCAGCTACCTGGAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCT
CACTCGTCGATGGACCTCCCGGTCCGGCGGTTCCTCAAGTAGCGGACCGA
 V  S  S  Y  L  E  G  Q  A  A  K  E  F  I  A  W  L>
____________________PRODUCT=GLP-1 ____________________>

        260
GGTGAAGGGCCGCGGC
CCACTTCCCGGCGCCG
  V  K  G  R  G>
___PRODUCT=_____>
```

Figure 5

IGF-I.GLP-1Gly8

```
                10        20        30        40        50
GAATTCCGCCCACCATGGGCAAGATCAGCAGCCTGCCCACCCAGCTGTTC
CTTAAGGCGGGTGGTACCCGTTCTAGTCGTCGGACGGGTGGGTCGACAAG
                M  G  K  I  S  S  L  P  T  Q  L  F>
               _______IGF-I SIGNAL PEPTIDE_________>

        60        70        80        90       100
                                                   *
AAGTGCTGCTTTTGTGACTTCCTGAAGGTGAAGATGCACACCATGAGCTC

TTCACGACGAAAACACTGAAGGACTTCCACTTCTACGTGTGGTACTCGAG
 K  C  C  F  C  D  F  L  K  V  K  M  H  T  M  S  S>
_________________IGF-I SIGNAL PEPTIDE_____________>

       110       120       130       140       150
CAGCCACCTGTTCTACCTGGCCCTGTGCCTGCTGACCTTCACCAGCTCCG
GTCGGTGGACAAGATGGACCGGGACACGGACGACTGGAAGTGGTCGAGGC
  S  H  L  F  Y  L  A  L  C  L  L  T  F  T  S  S>
_________________IGF-I SIGNAL PEPTIDE_____________>

       160       170       180       190       200
                                                   *
CCACAGCCCACGGCGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTG
GGTGTCGGGTGCCGCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGAC
A  T  A>
_________>H  G  E  G  T  F  T  S  D  V  S  S  Y  L>
          _________________GLP-1GLY8_________________>

       210       220       230       240       250
GAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
CTCCCGGTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
 E  G  Q  A  A  K  E  F  I  A  W  L  V  K  G  R  G>
_____________________GLP-1GLY8_____________________>
```

Figure 6

Preproglucagon.GLP-1Gly8

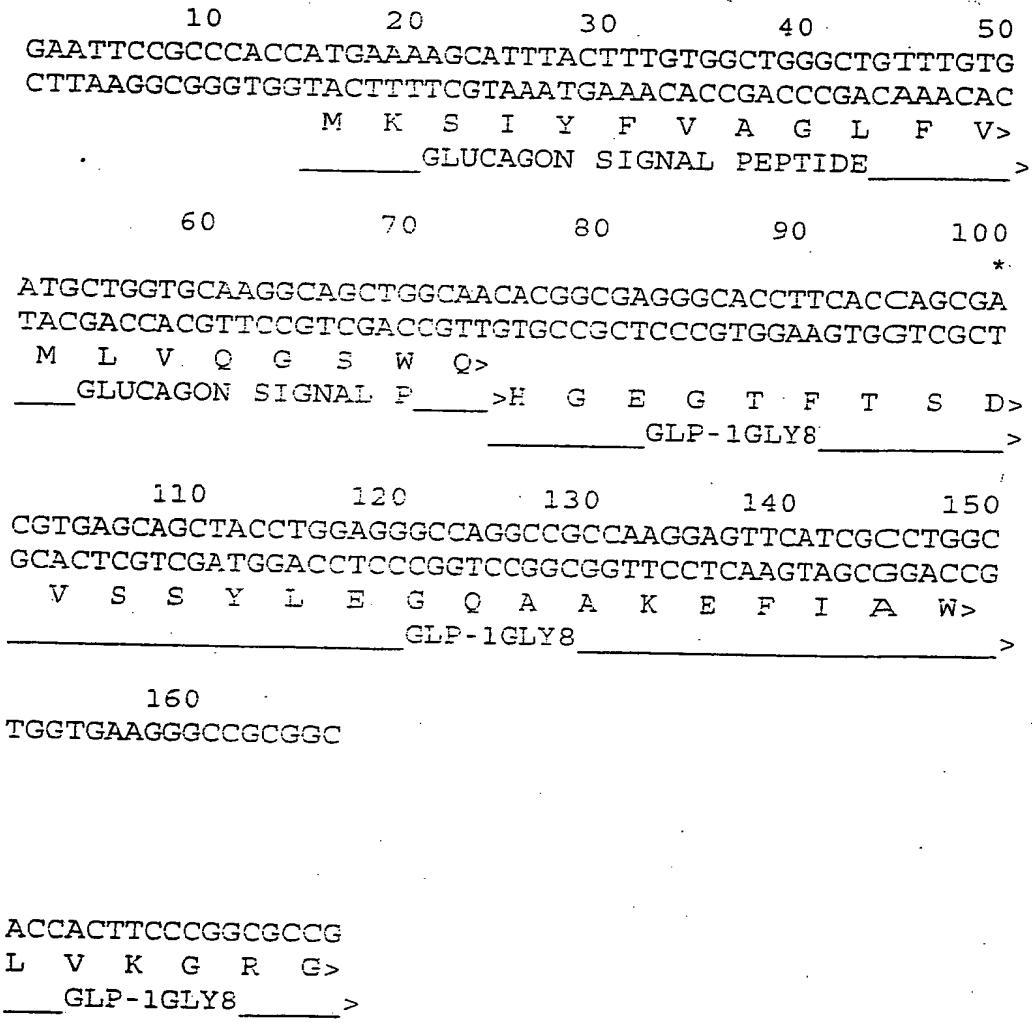


Figure 7

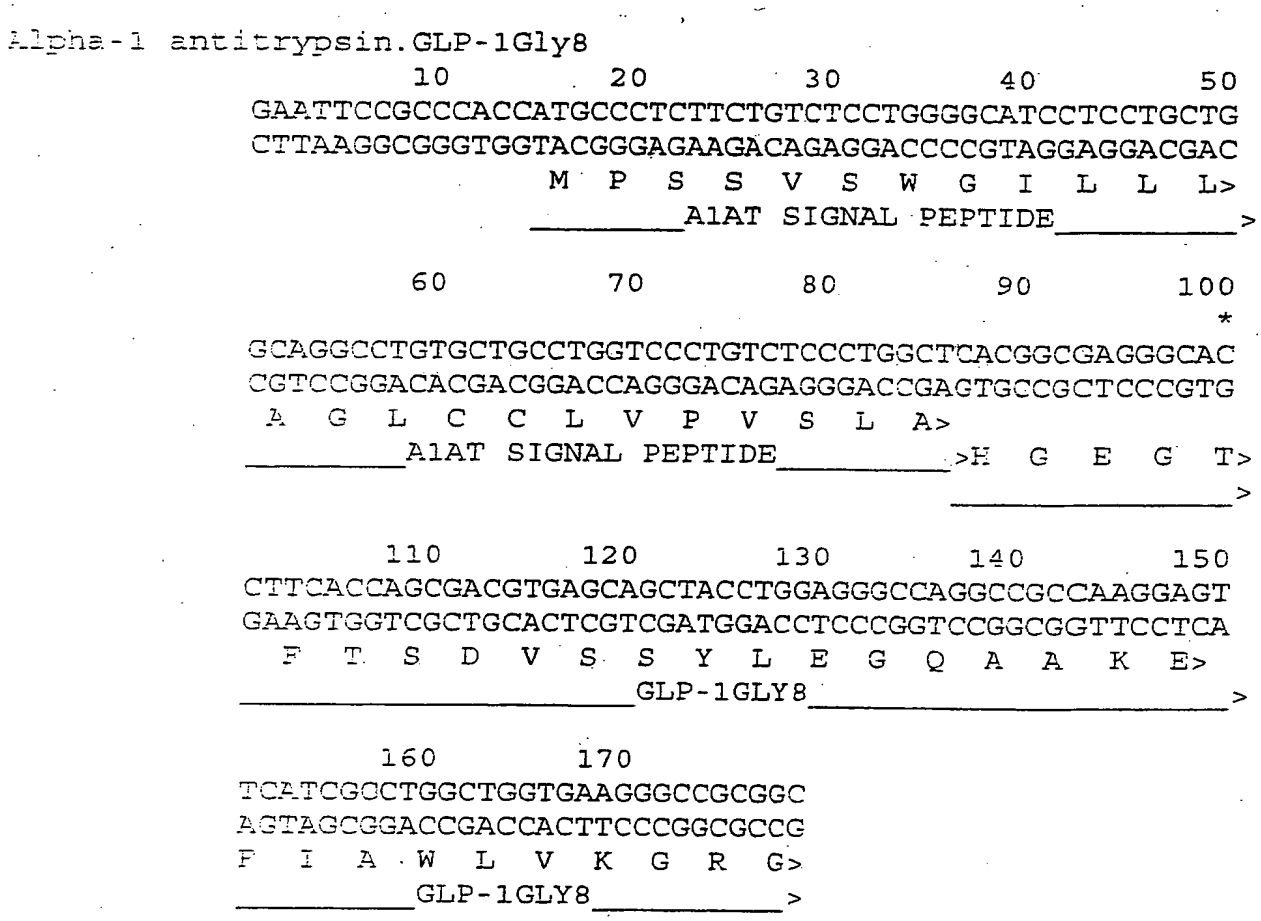

Alpha-1 antitrypsin.GLP-1Gly8
10 20 30 40 50
GAATTCCGCCCACCATGCCCTCTTCTGTCTCCTGGGGCATCCTCCTGCTG
CTTAAGGCGGGTGGTACGGGAGAAGACAGAGGACCCCGTAGGAGGACGAC
M P S S V S W G I L L L>
A1AT SIGNAL PEPTIDE>
60 70 80 90 100
GCAGGCCTGTGCTGCCTGGTCCCTGTCTCCCTGGCTCACGGCGAGGGCAC
CGTCCGGACACGACGGACCAGGGACAGAGGGACCGAGTGCCGCTCCCGTG
A G L C C L V P V S L A>
A1AT SIGNAL PEPTIDE>H G E G T>
110 120 130 140 150
CTTCACCAGCGACGTGAGCAGCTACCTGGAGGGCCAGGCCGCCAAGGAGT
GAAGTGGTCGCTGCACTCGTCGATGGACCTCCCGGTCCGGCGGTTCCTCA
F T S D V S S Y L E G Q A A K E>
GLP-1GLY8>
160 170
TCATCGCCTGGCTGGTGAAGGGCCGCGGC
AGTAGCGGACCGACCACTTCCCGGCGCCG
F I A W L V K G R G>
GLP-1GLY8>

Figure 8

Factor IX.GLP-1Gly8

```
                10         20         30         40         50
GAATTCCGCCCACCATGCAGAGAGTGAACATGATCATGGCAGAATCCCCA
CTTAAGGCGGGTGGTACGTCTCTCACTTGTACTAGTACCGTCTTAGGGGT
                M  Q  R  V  N  M  I  M  A  E  S  P>
                _____________PRO-FIX_____________>

          60         70         80         90        100
                                                      *
GGCCTGATCACCATCTGCCTCCTGGGATACCTCCTGTCTGCTGAGTGCAC
CCGGACTAGTGGTAGACGGAGGACCCTATGGAGGACAGACGACTCACGTG
 G  L  I  T  I  C  L  L  G  Y  L  L  S  A  E  C  T>
_________________________PRO-FIX_________________________>

        110        120        130        140        150
AGTGTTCCTGGACCATGAGAATGCCAACAAGATTCTGAACAGACCCAAGA
```

```
TCACAAGGACCTGGTACTCTTACGGTTGTTCTAAGACTTGTCTGGGTTCT
  V  F  L  D  H  E  N  A  N  K  I  L  N  R  P  K>
_________________________PRO-FIX_________________________>

        160        170        180        190        200
                                                      *
GGCATGGGGAGGGCACCTTCACCAGCGACGTGAGCAGCTACCTGGAGGGC
CCGTACCCCTCCCGTGGAAGTGGTCGCTGCACTCGTCGATGGACCTCCCG
R>
__>H  G  E  G  T  F  T  S  D  V  S  S  Y  L  E  G>
  ____________________GLP-1GLY-8 ____________________>

        210        220        230        240
CAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
GTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
 Q  A  A  K  E  F  I  A  W  L  V  K  G  R  G>
_________________GLP-1GLY-8 _________________>
```

Figure 9

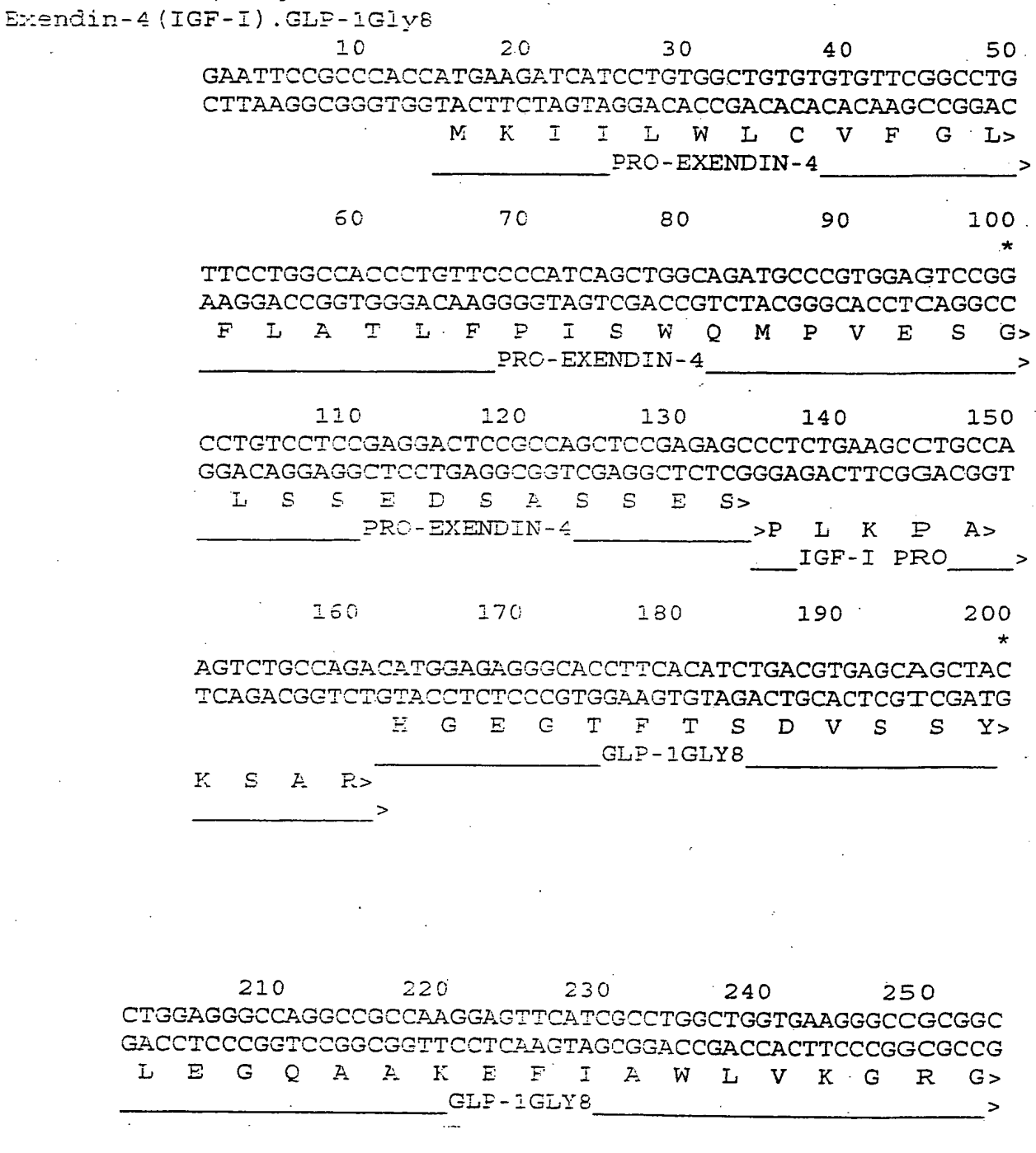

Exendin-4(IGF-I).GLP-1Gly8
10 20 30 40 50
GAATTCCGCCCACCATGAAGATCATCCTGTGGCTGTGTGTGTTCGGCCTG
CTTAAGGCGGGTGGTACTTCTAGTAGGACACCGACACACACAAGCCGGAC
M K I I L W L C V F G L>
PRO-EXENDIN-4
60 70 80 90 100
TTCCTGGCCACCCTGTTCCCCATCAGCTGGCAGATGCCCGTGGAGTCCGG
AAGGACCGGTGGGACAAGGGGTAGTCGACCGTCTACGGGCACCTCAGGCC
F L A T L F P I S W Q M P V E S G>
PRO-EXENDIN-4
110 120 130 140 150
CCTGTCCTCCGAGGACTCCGCCAGCTCCGAGAGCCCTCTGAAGCCTGCCA
GGACAGGAGGCTCCTGAGGCGGTCGAGGCTCTCGGGAGACTTCGGACGGT
L S S E D S A S S E S>
PRO-EXENDIN-4
>P L K P A>
IGF-I PRO
160 170 180 190 200
AGTCTGCCAGACATGGAGAGGGCACCTTCACATCTGACGTGAGCAGCTAC
TCAGACGGTCTGTACCTCTCCCGTGGAAGTGTAGACTGCACTCGTCGATG
H G E G T F T S D V S S Y>
GLP-1GLY8
K S A R>
210 220 230 240 250
CTGGAGGGCCAGGCCGCCAAGGAGTTCATCGCCTGGCTGGTGAAGGGCCGCGGC
GACCTCCCGGTCCGGCGGTTCCTCAAGTAGCGGACCGACCACTTCCCGGCGCCG
L E G Q A A K E F I A W L V K G R G>
GLP-1GLY8

Figure 10

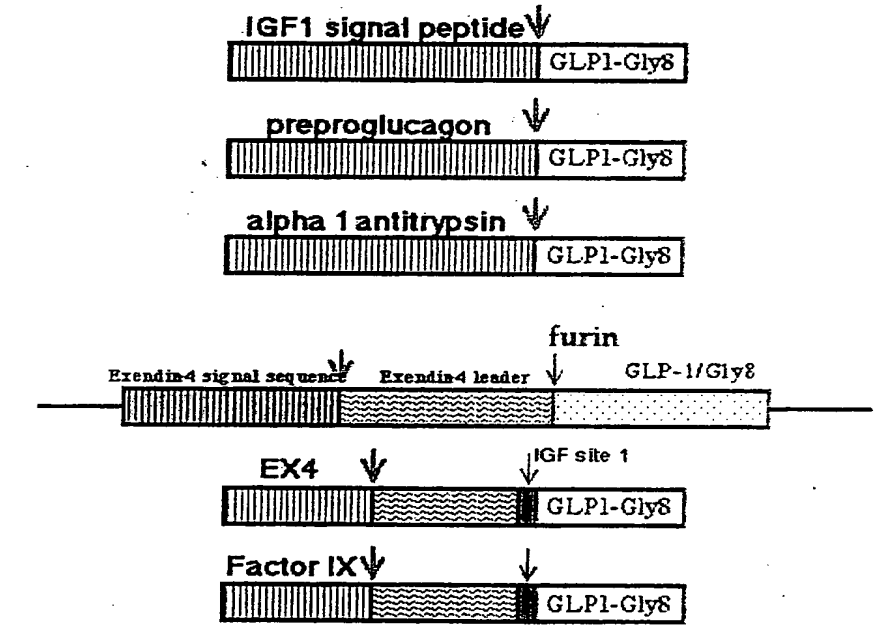

IGF1 signal peptide
GLP1-Gly8
preproglucagon
GLP1-Gly8
alpha 1 antitrypsin
GLP1-Gly8
furin
Exendin-4 signal sequence
Exendin-4 leader
GLP-1/Gly8
EX4
IGF site 1
GLP1-Gly8
Factor IX
GLP1-Gly8
predicted signal peptidase cleavage site
predicted prohormone convertase cleavage site

Figure 11

GLP-1 Expression Levels in the Supernatant of Transfected 293 Cells

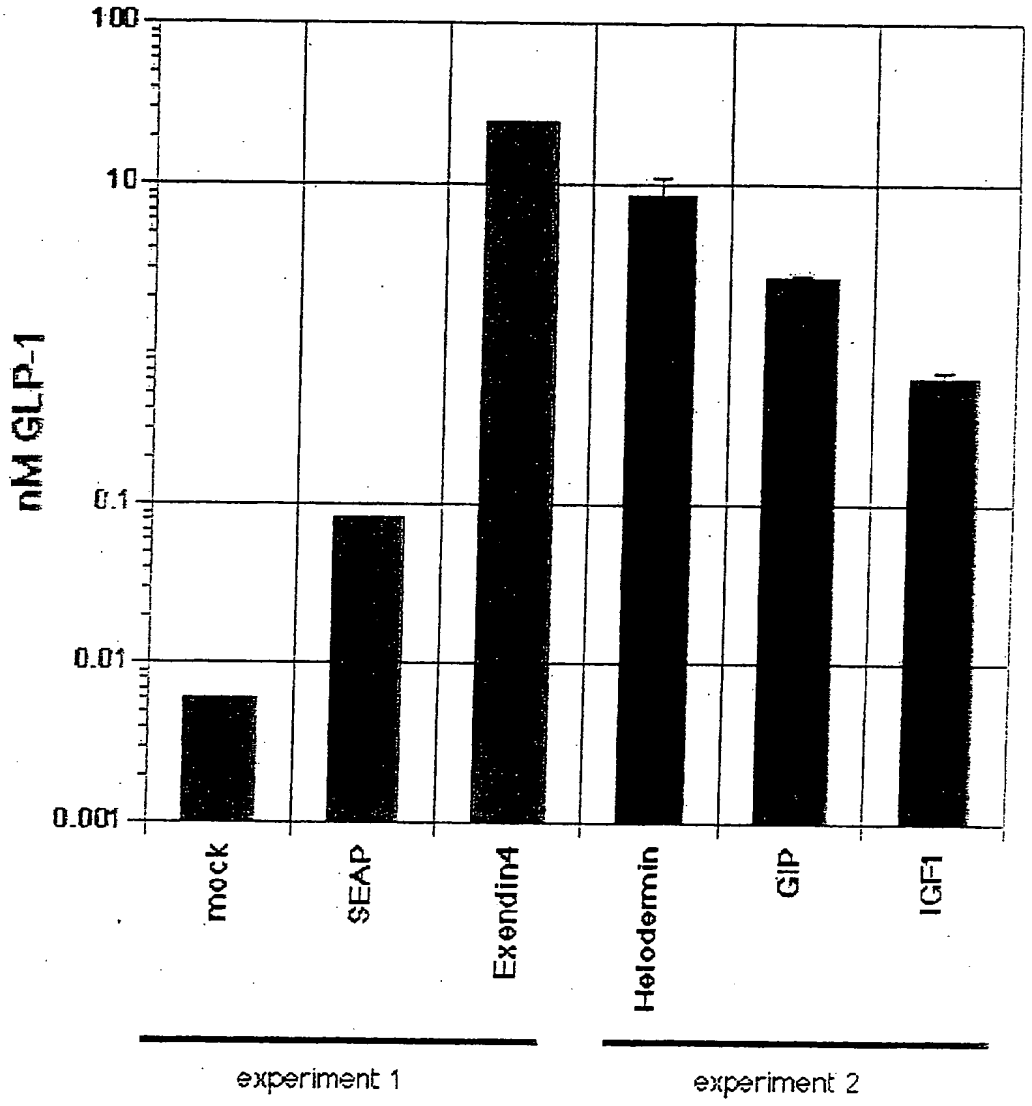


Figure 12

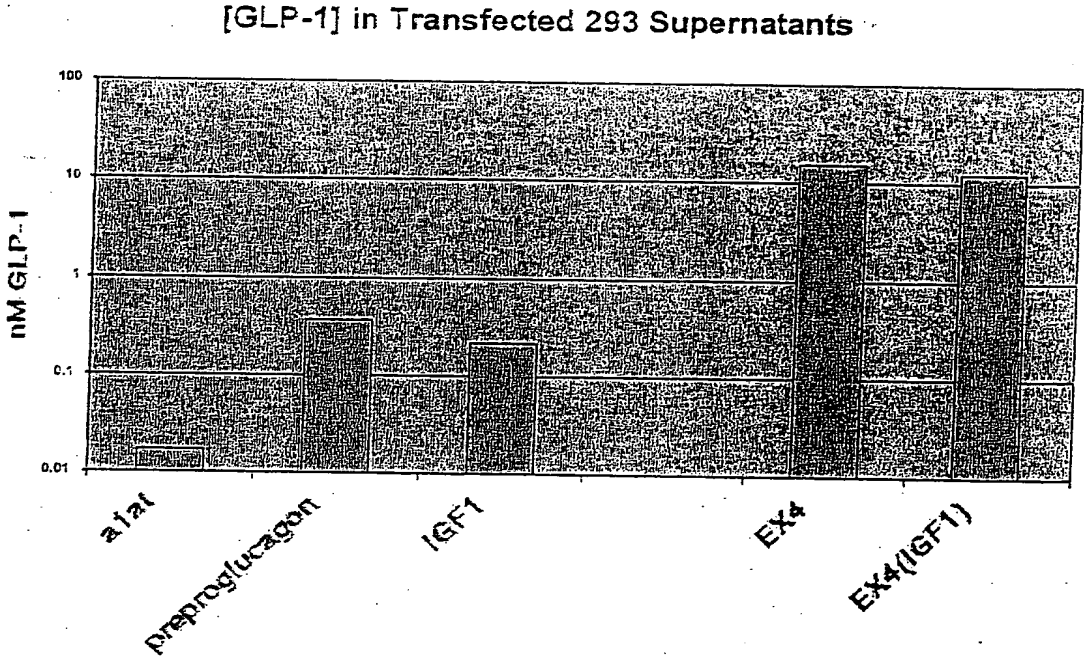

[GLP-1] in Transfected 293 Supernatants
nM GLP-1
100
10
1
0.1
0.01
alat
preproglucagon
IGF1
EX4
EX4(IGF1)

Figure 13

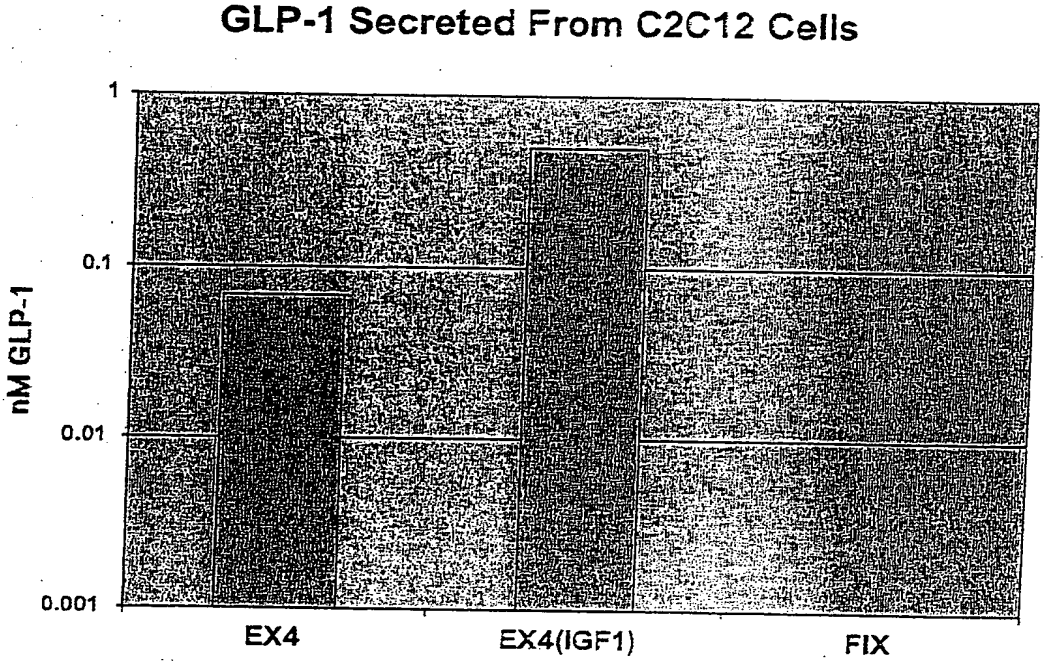

GLP-1 Secreted From C2C12 Cells
nM GLP-1
1
0.1
0.01
0.001
EX4
EX4(IGF1)
FIX

Figure 14

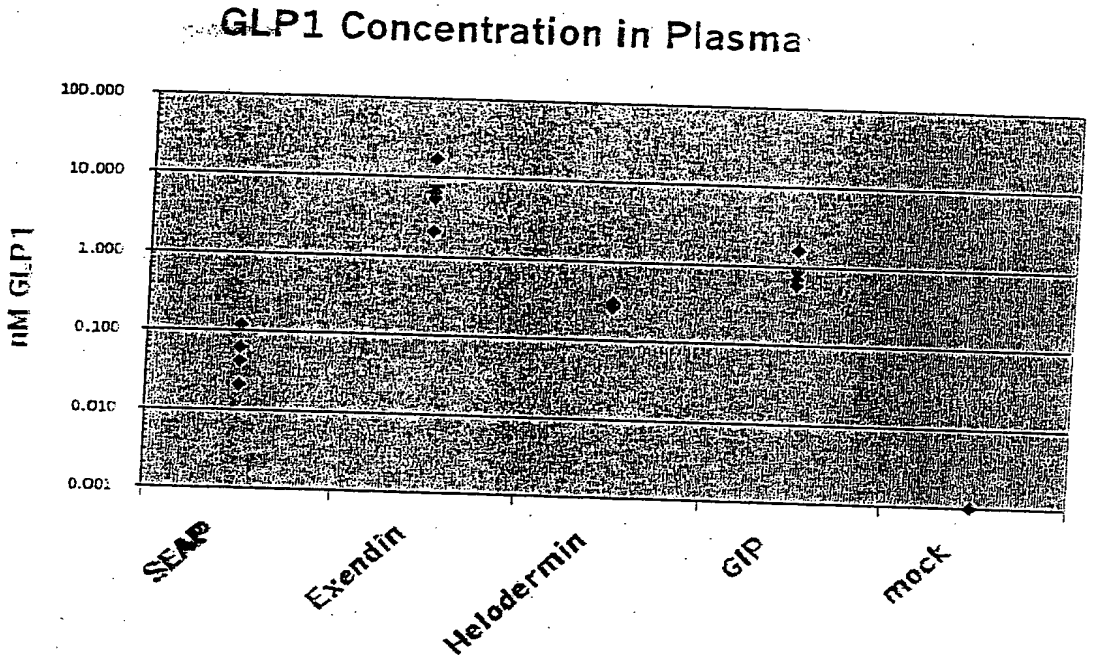

GLP1 Concentration in Plasma
100.000
10.000
1.000
0.100
0.010
0.001
nM GLP1
SEAP
Exendin
Heloderrmin
GIP
mock

Figure 15

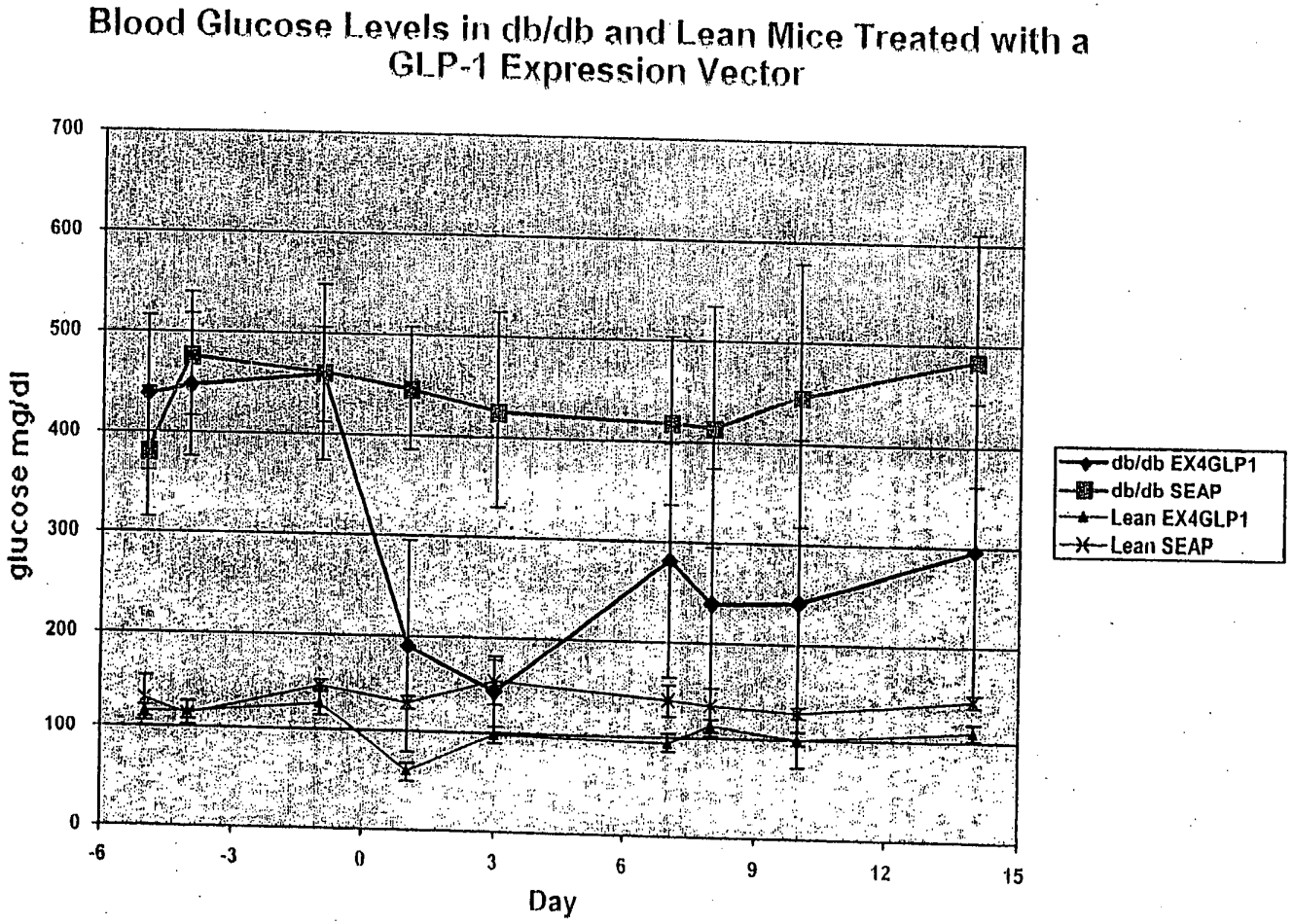

Blood Glucose Levels in db/db and Lean Mice Treated with a GLP-1 Expression Vector
glucose mg/dl
700
600
500
400
300
200
100
0
-6
-3
0
3
6
9
12
15
Day
db/db EX4GLP1
db/db SEAP
Lean EX4GLP1
Lean SEAP

Figure 16

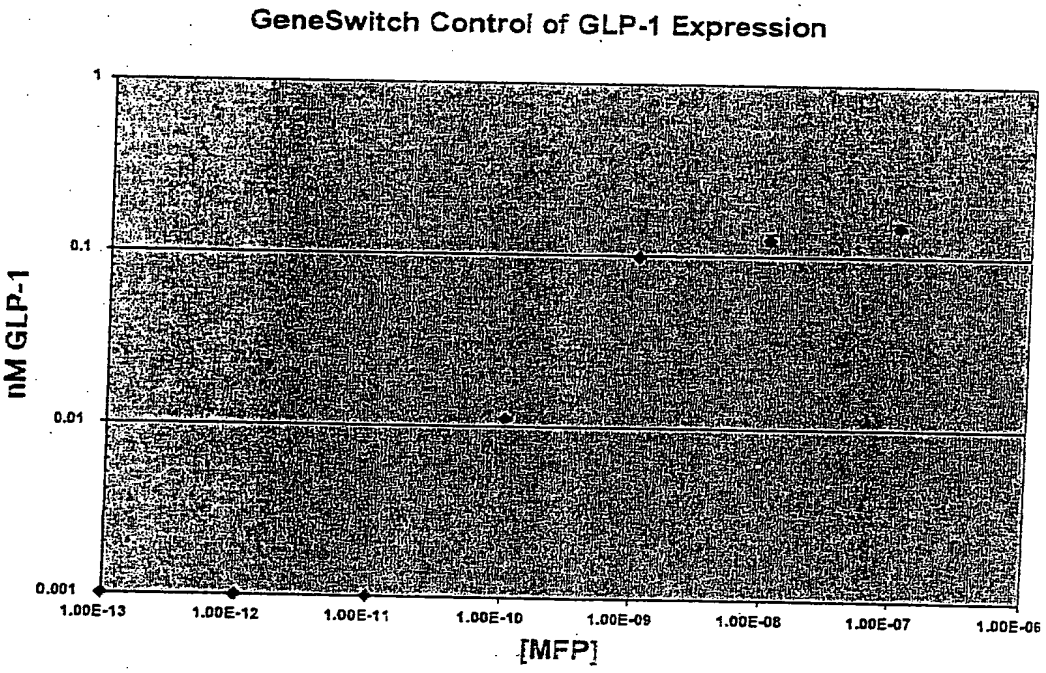

GeneSwitch Control of GLP-1 Expression
nM GLP-1
1
0.1
0.01
0.001
1.00E-13
1.00E-12
1.00E-11
1.00E-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
[MFP]

Figure 17

Examples of Modified GLP-1

His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys (SEQ ID NO:23)

His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35} (SEQ ID NO: 24)

His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg (SEQ ID NO:25)

His^{7}-Val-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:26)

His^{7}-Ala-Gln-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:27)

His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Thr-Ser-Lys-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37} (SEQ ID NO:28)

His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Lys-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:29)

^{His7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-D-GLn^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:30)

Figure 18A

Asp-Glu-Phe-Glu-Arg-His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:31)

Glu-Phe-Glu-Arg-His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:32)

Arg-His^{7}-Ala-Glu-Gly^{10}-Thr-Phe-Thr-Ser-Asp^{15}-Val-Ser-Ser-Tyr-Leu^{20}-Glu-Gly-Gln-Ala-Ala^{25}-Lys-Glu-Phe-Ile-Ala^{30}-Trp-Leu-Val-Lys-Gly^{35}-Arg-Gly^{37}-COOH (SEQ ID NO:33)

Figure 18B

Figure 19

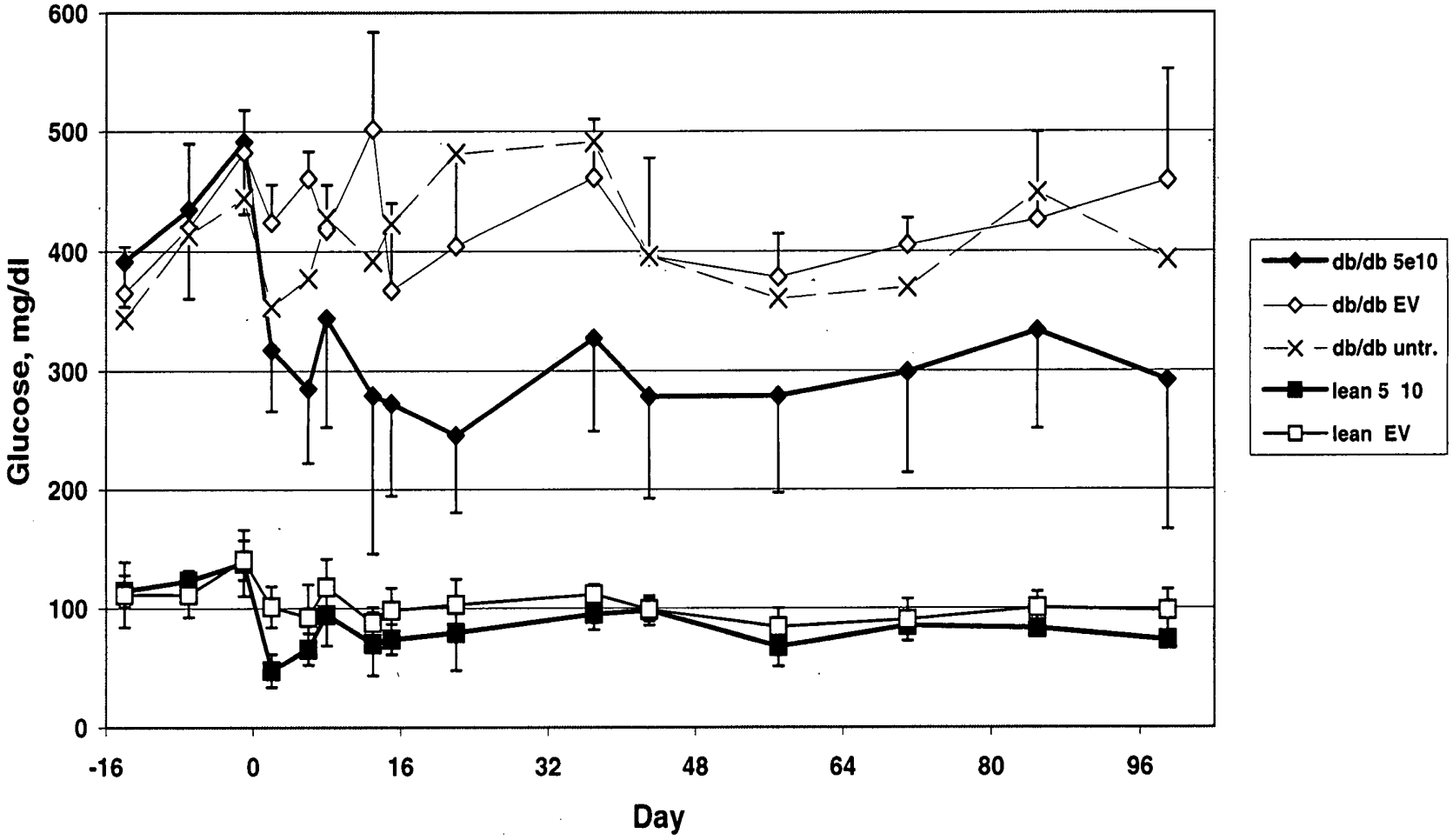

600
500
400
300
200
100
0
Glucose, mg/dl
-16
0
16
32
48
64
80
96
Day
db/db 5e10
db/db EV
db/db untr.
lean 5 10
lean EV

Figure 20

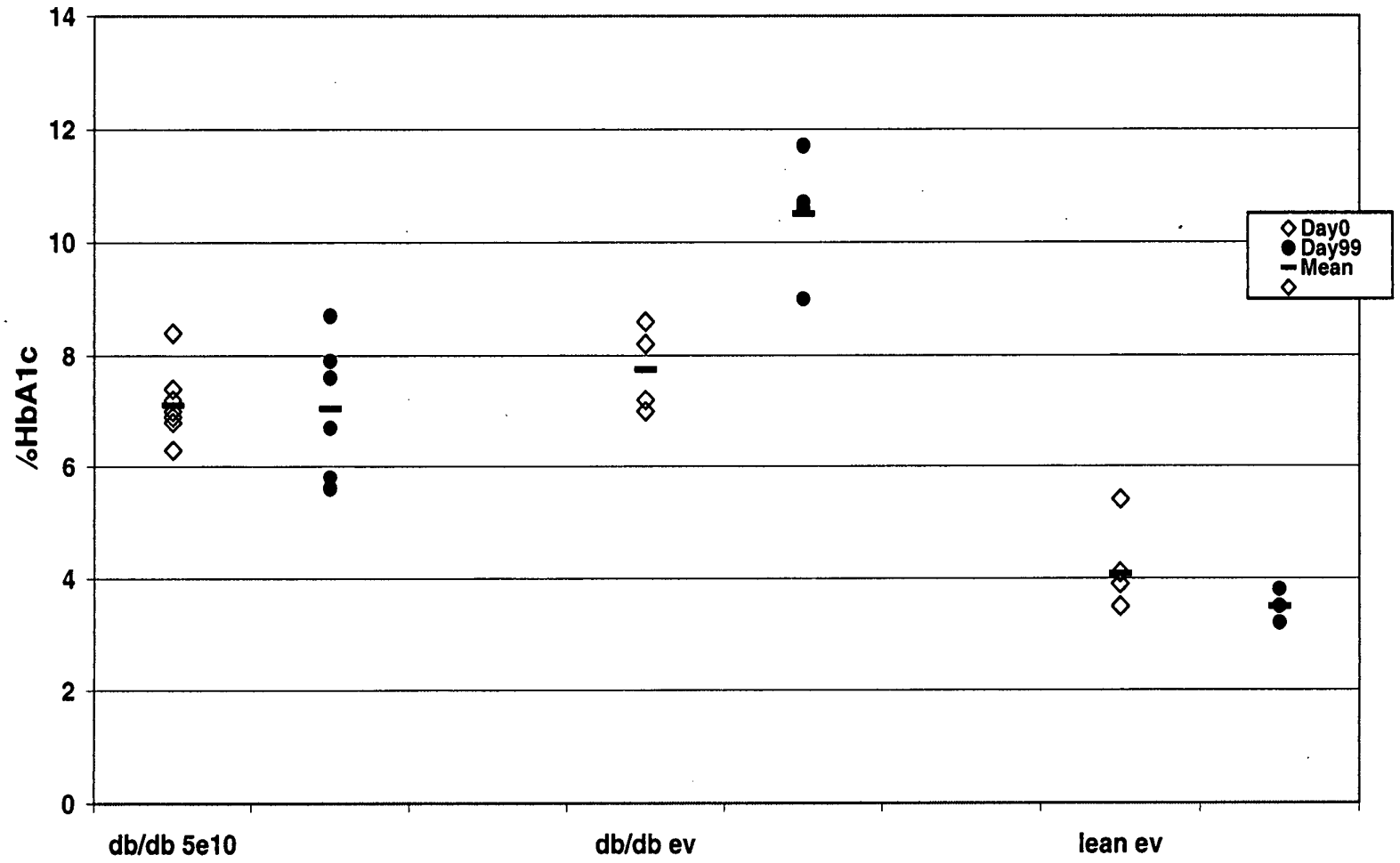


Figure 21

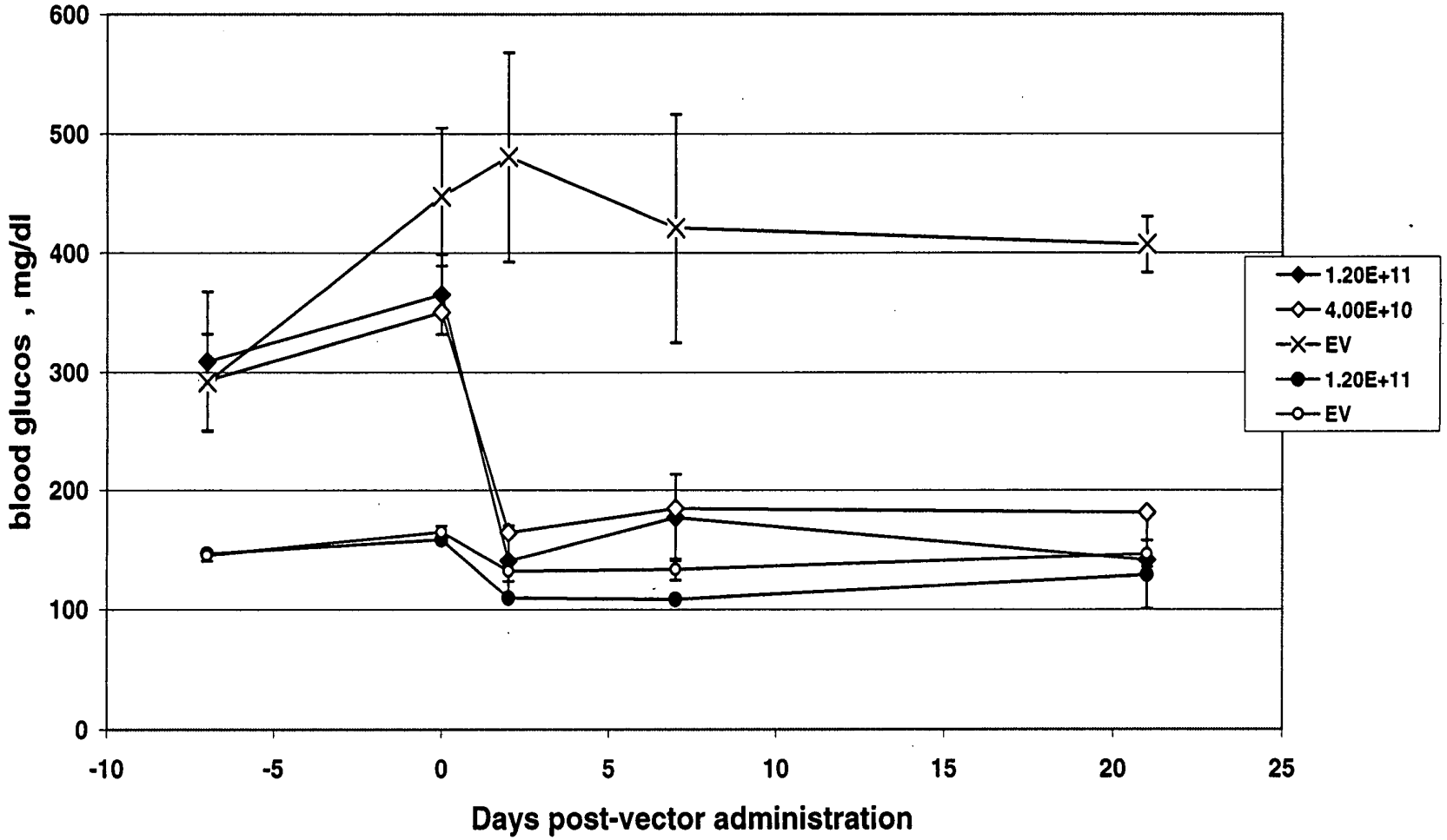

600
500
400
300
200
100
0
blood glucos , mg/dl
-10
-5
0
5
10
15
20
25
Days post-vector administration
1.20E+11
4.00E+10
EV
1.20E+11
EV

Figure 22

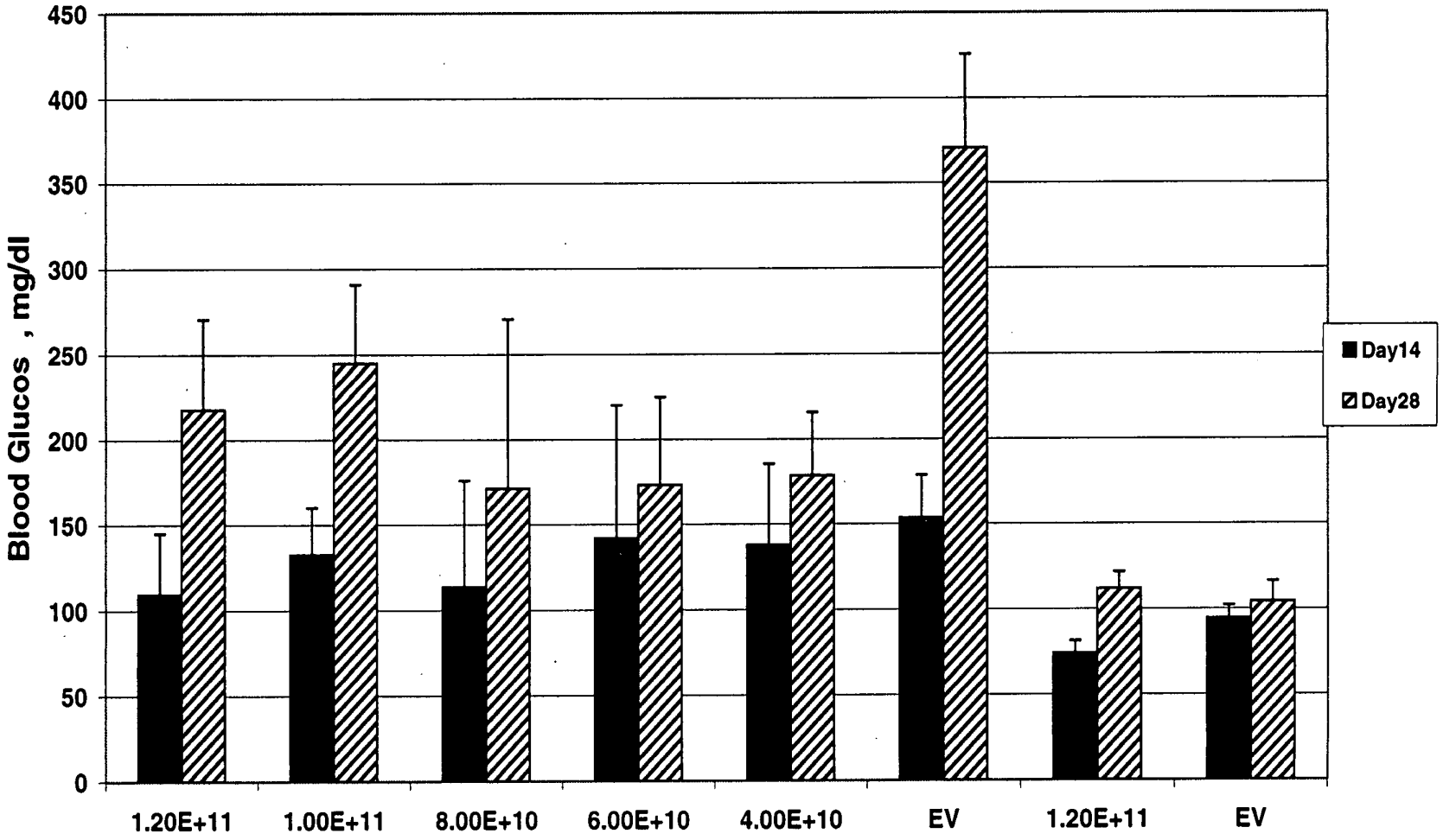

Blood Glucos , mg/dl
450
400
350
300
250
200
150
100
50
0
1.20E+11
1.00E+11
8.00E+10
6.00E+10
4.00E+10
EV
1.20E+11
EV
Day14
Day28

Figure 23

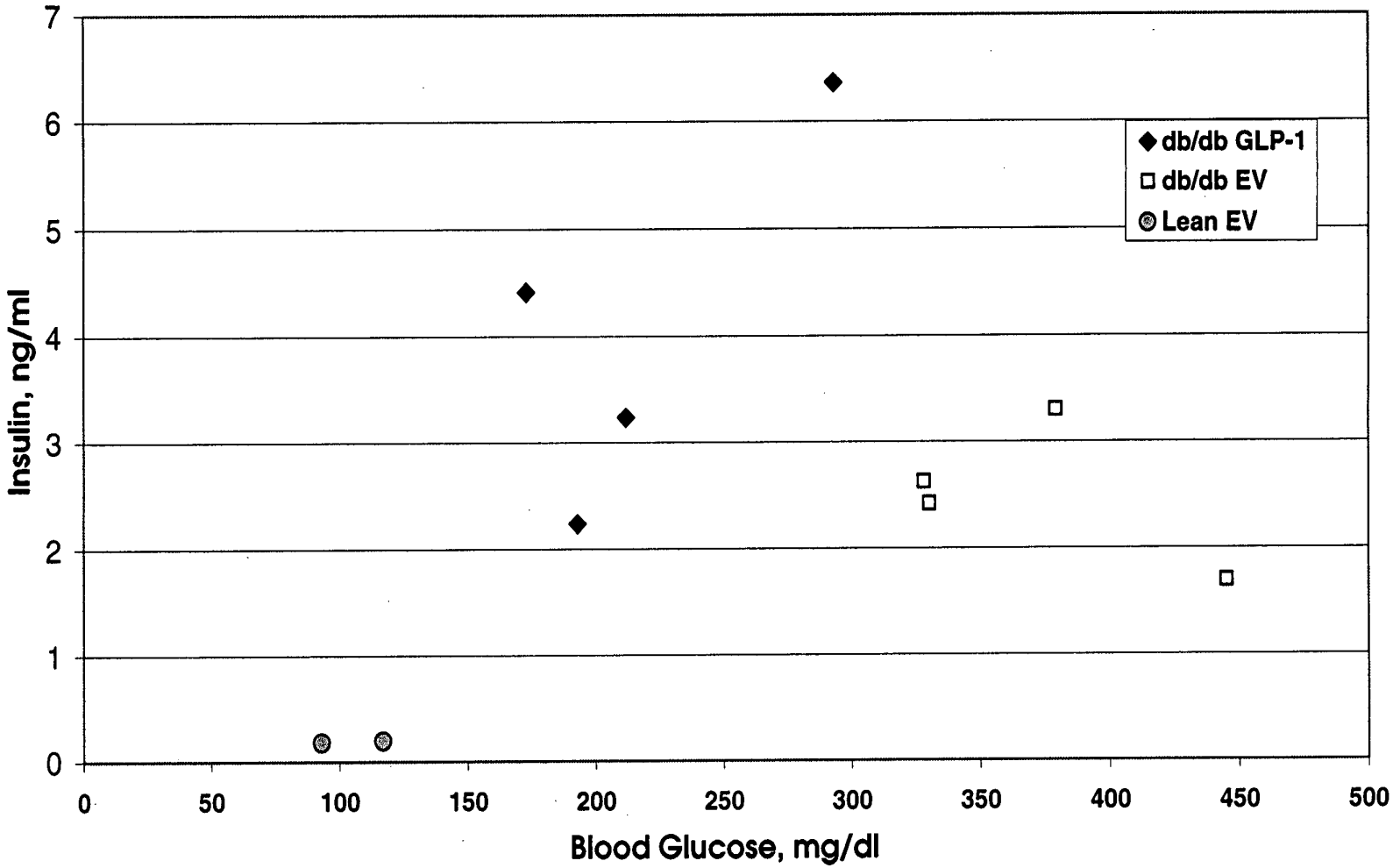

Insulin, ng/ml
Blood Glucose, mg/dl
db/db GLP-1
db/db EV
Lean EV
0
1
2
3
4
5
6
7
0
50
100
150
200
250
300
350
400
450
500

Figure 24

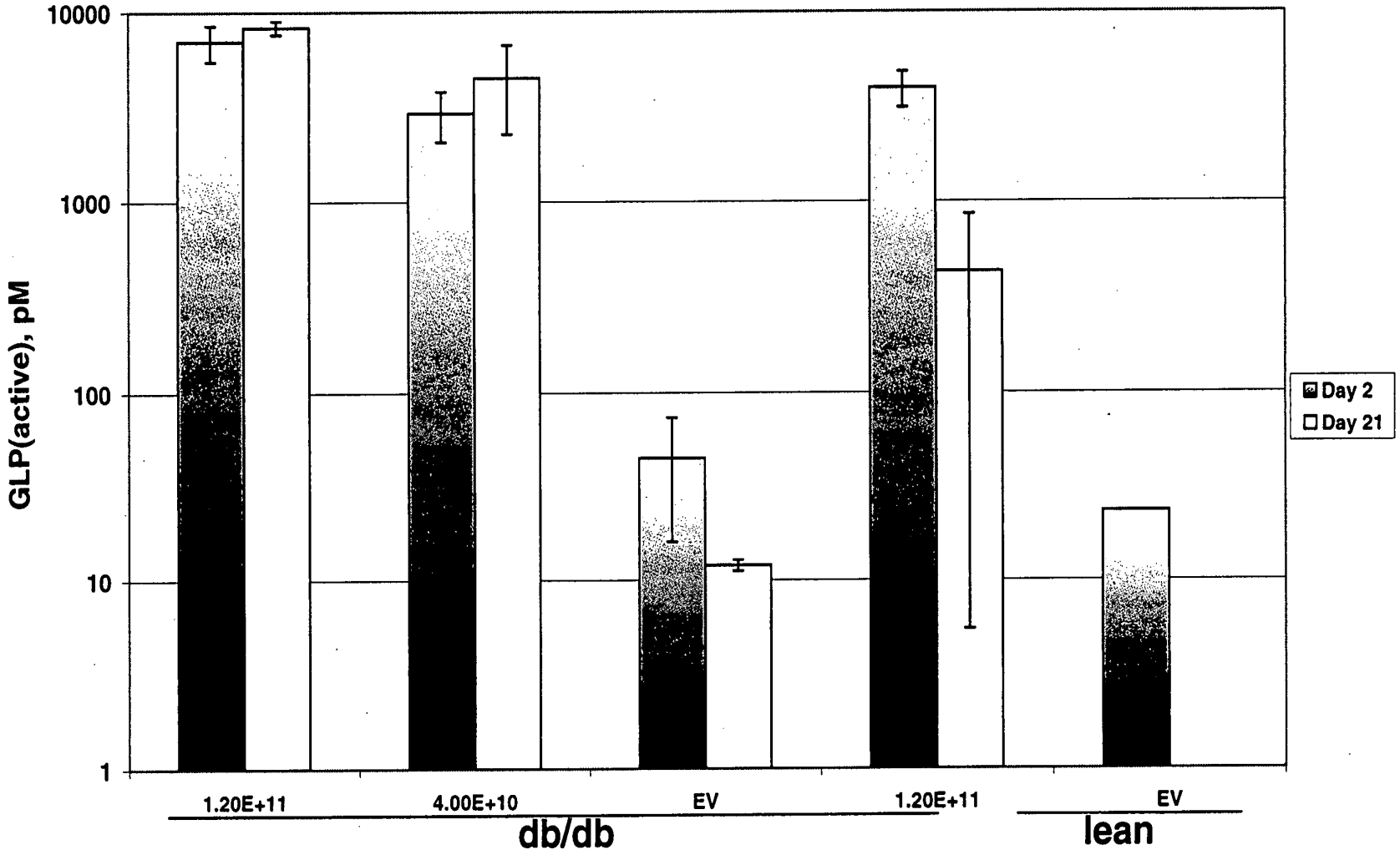

10000
1000
100
10
1
GLP(active), pM
Day 2
Day 21
1.20E+11
4.00E+10
EV
1.20E+11
EV
db/db
lean

Figure 25

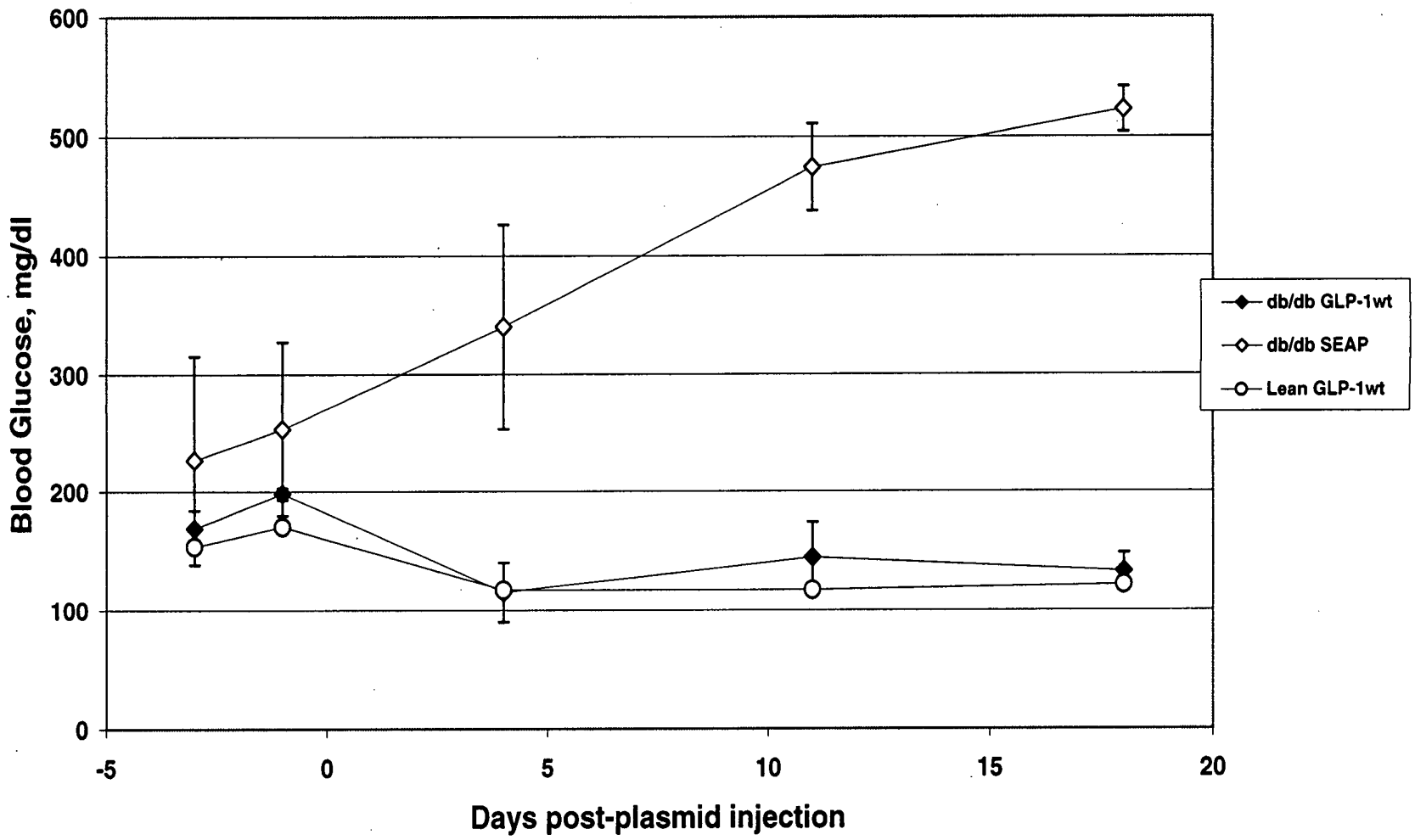

600
500
400
300
200
100
0
Blood Glucose, mg/dl
-5
0
5
10
15
20
Days post-plasmid injection
db/db GLP-1wt
db/db SEAP
Lean GLP-1wt

Figure 26

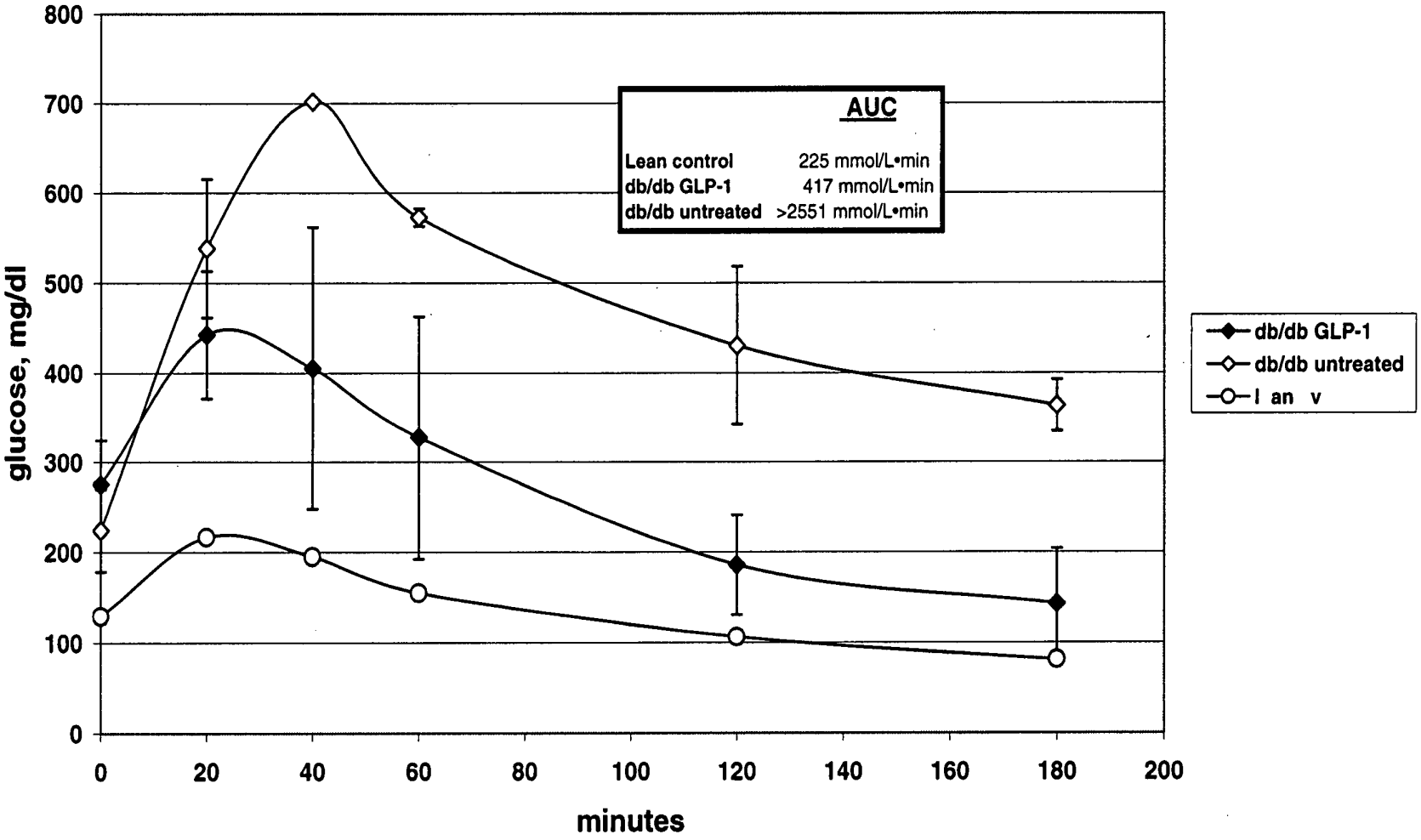

800
700
600
500
400
300
200
100
0
glucose, mg/dl
0
20
40
60
80
100
120
140
160
180
200
minutes
AUC
Lean control 225 mmol/L•min
db/db GLP-1 417 mmol/L•min
db/db untreated >2551 mmol/L•min
db/db GLP-1
db/db untreated
l an v

Figure 27

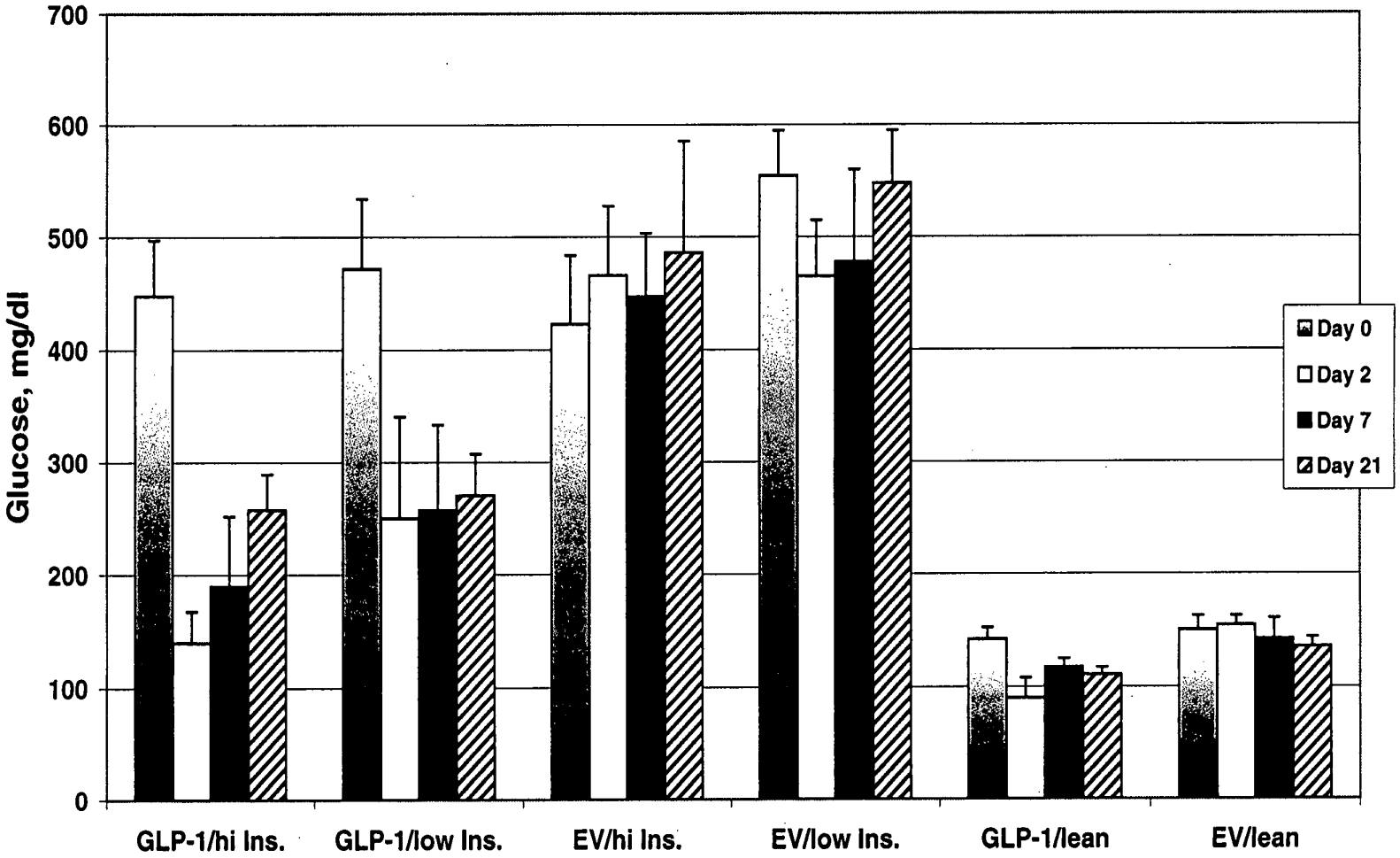

700
600
500
400
300
200
100
0
Glucose, mg/dl
Day 0
Day 2
Day 7
Day 21
GLP-1/hi Ins.
GLP-1/low Ins.
EV/hi Ins.
EV/low Ins.
GLP-1/lean
EV/lean

Figure 28

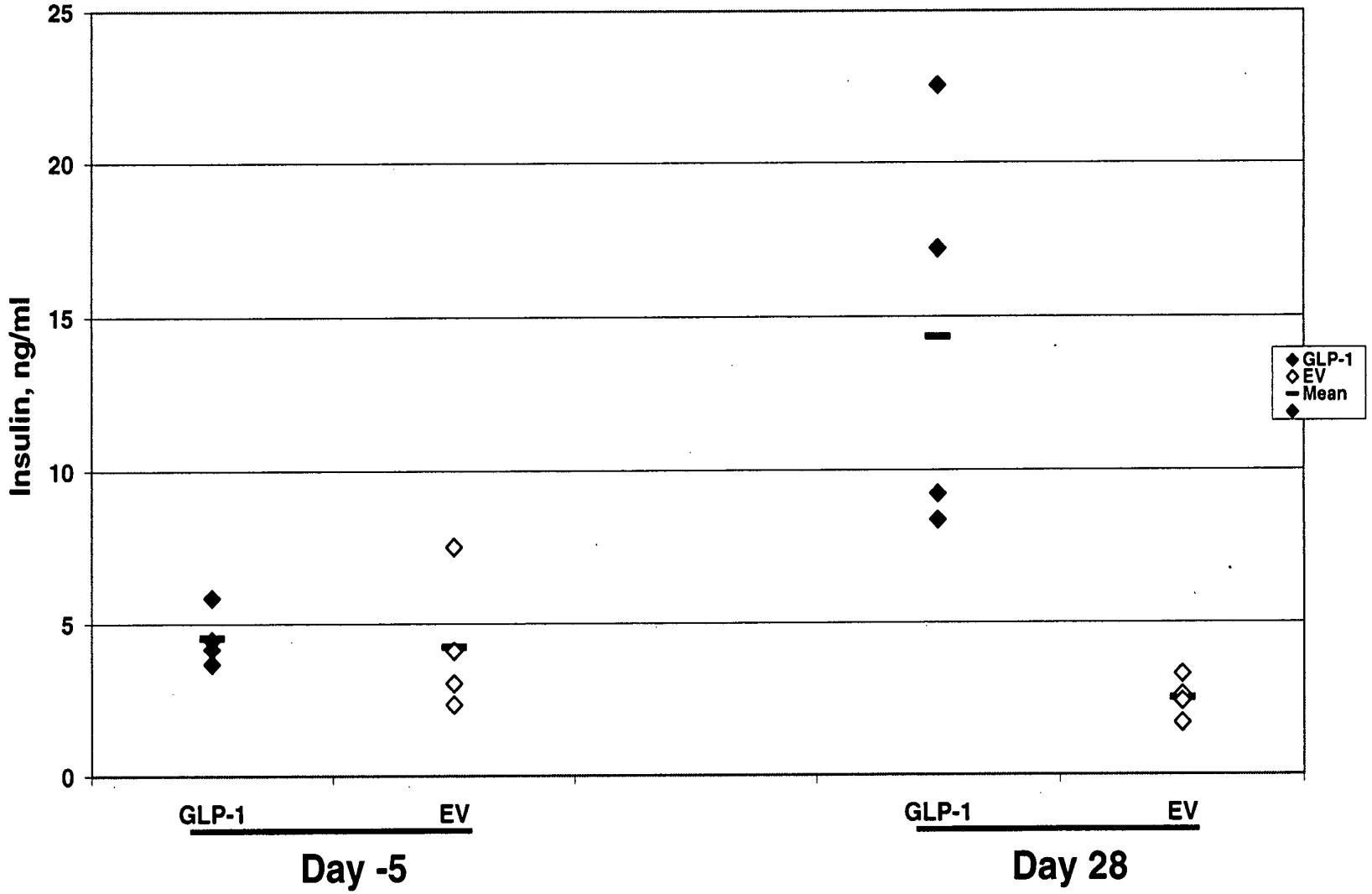

25
20
15
10
5
0
Insulin, ng/ml
GLP-1
EV
Mean
GLP-1
EV
GLP-1
EV
Day -5
Day 28

Figure 29

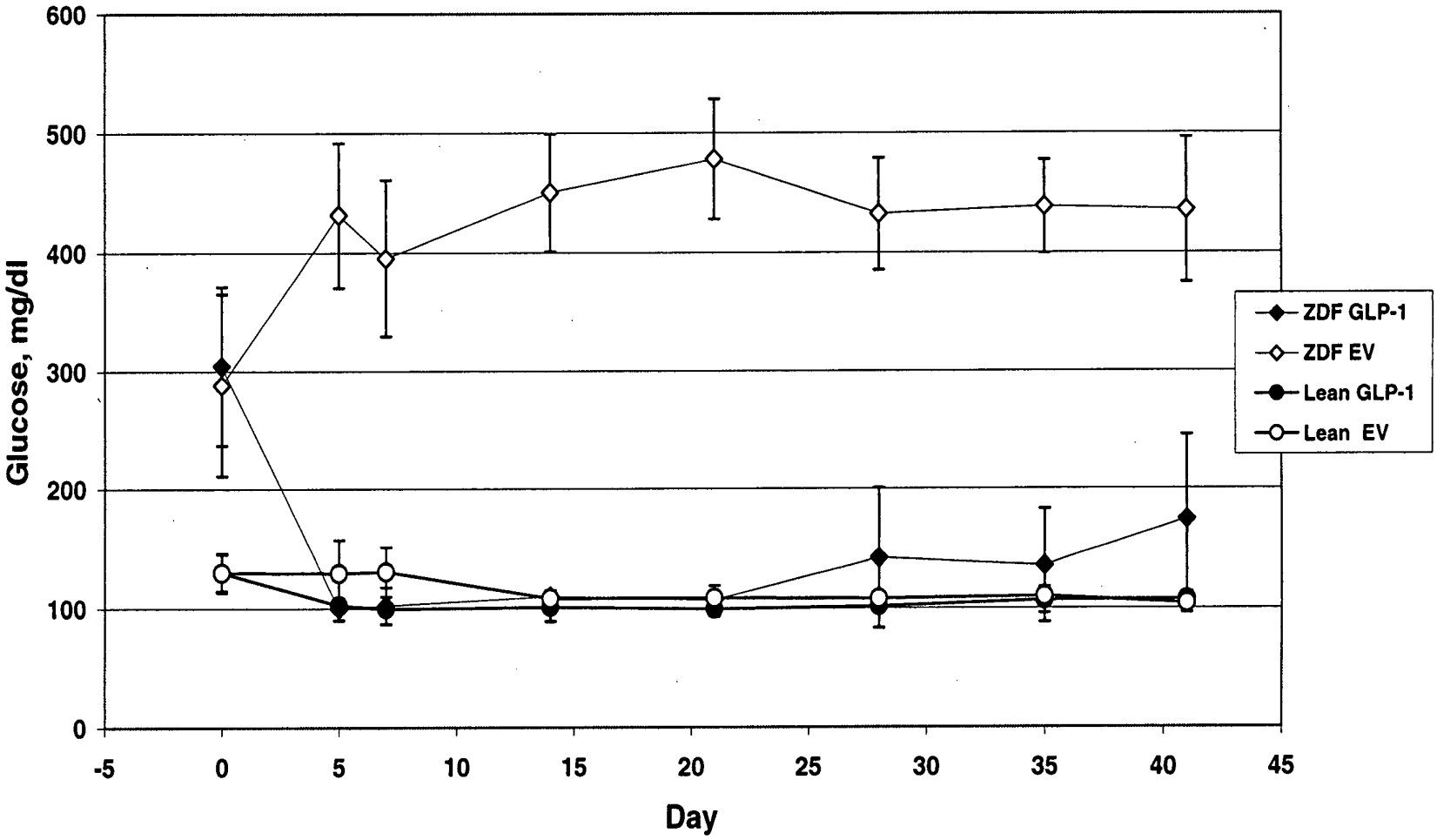

600
500
400
300
200
100
0
Glucose, mg/dl
-5
0
5
10
15
20
25
30
35
40
45
Day
ZDF GLP-1
ZDF EV
Lean GLP-1
Lean EV

Figure 30

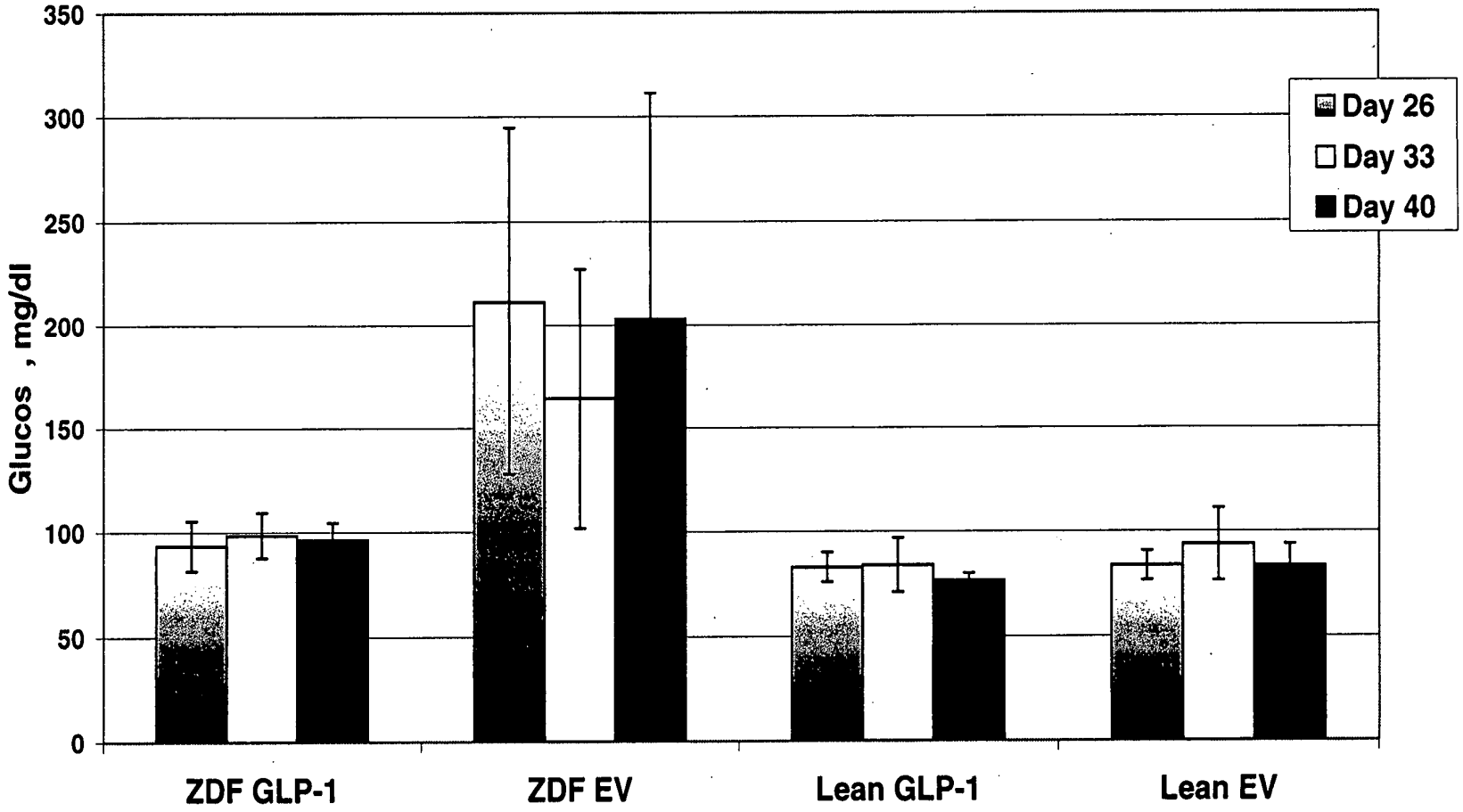

350
300
250
200
150
100
50
0
Glucos , mg/dl
Day 26
Day 33
Day 40
ZDF GLP-1
ZDF EV
Lean GLP-1
Lean EV

Figure 31

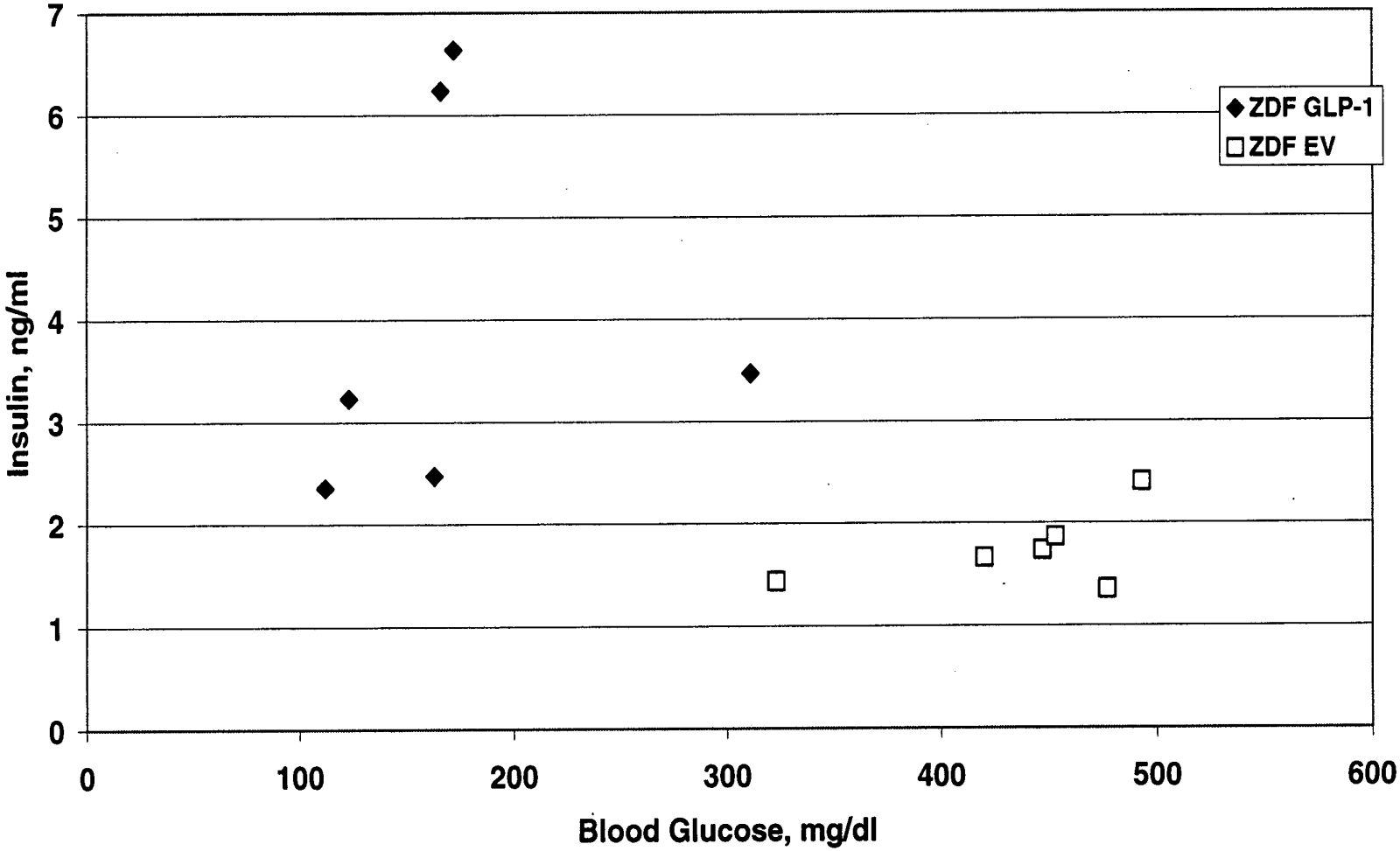

Insulin, ng/ml
0
1
2
3
4
5
6
7
0
100
200
300
400
500
600
Blood Glucose, mg/dl
ZDF GLP-1
ZDF EV

Figure 32

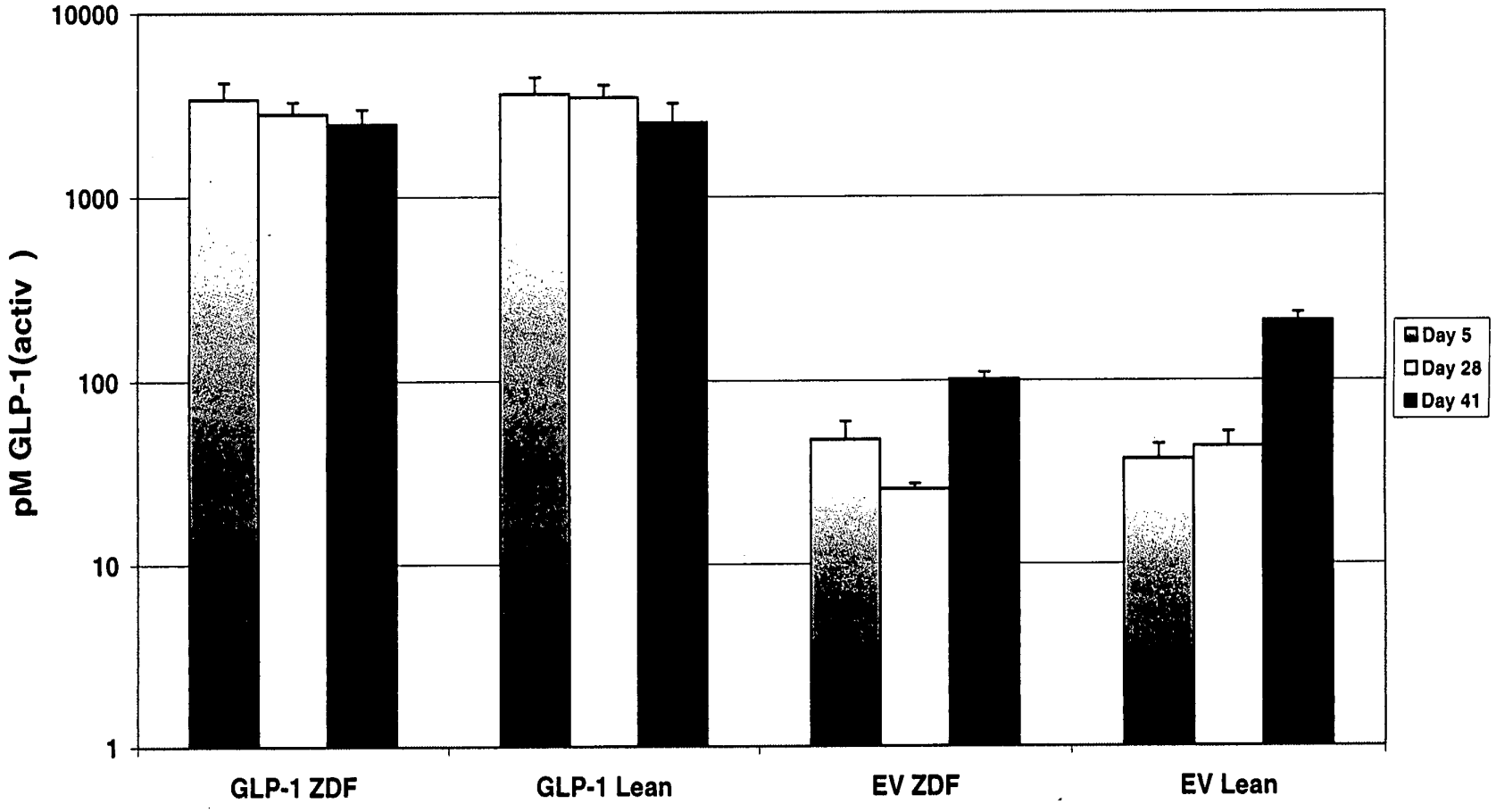

10000
1000
100
10
1
pM GLP-1(activ)
GLP-1 ZDF
GLP-1 Lean
EV ZDF
EV Lean
Day 5
Day 28
Day 41

Figure 33

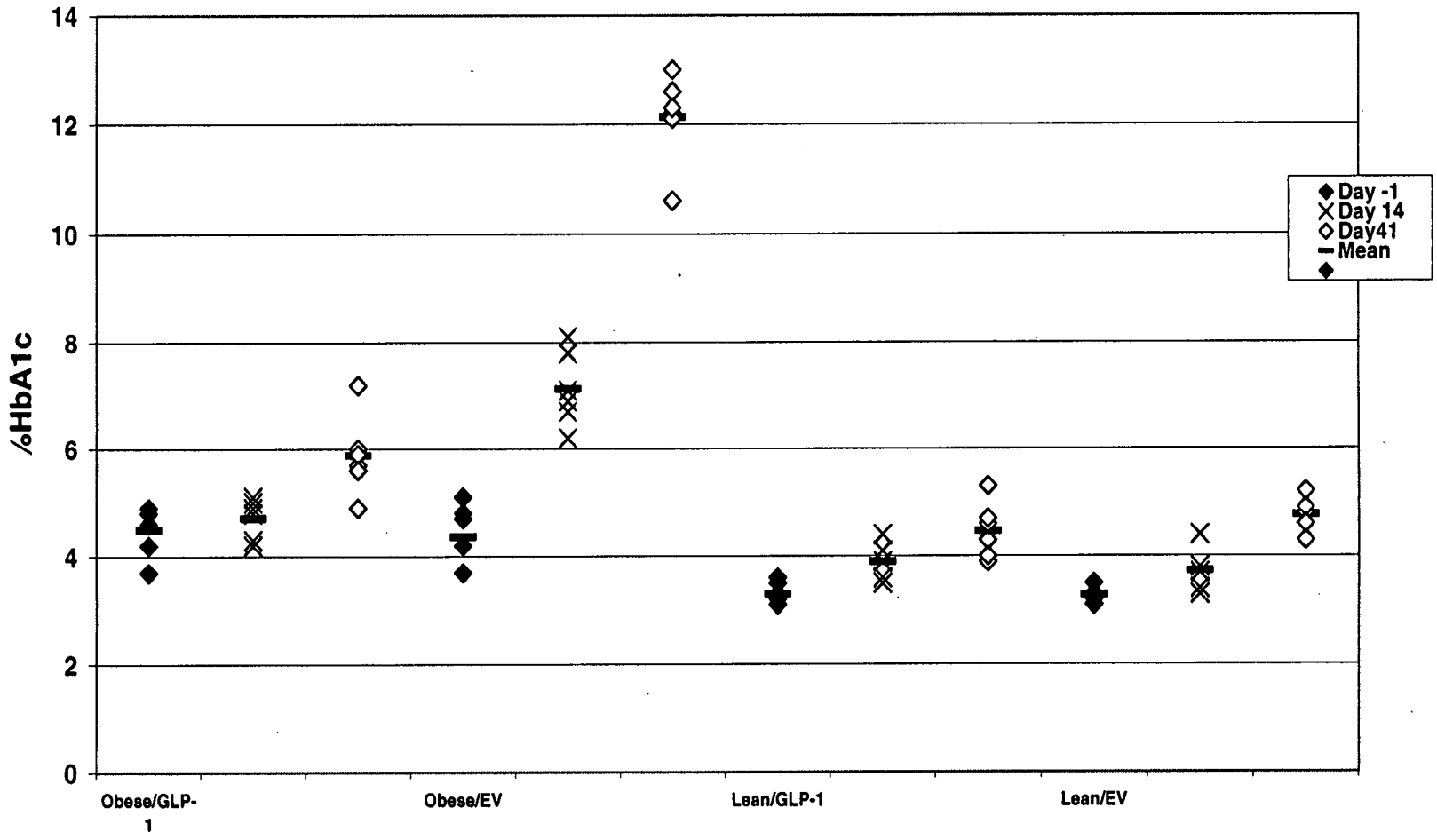

14
12
10
8
6
4
2
0
%HbA1c
Day -1
Day 14
Day41
Mean
Obese/GLP-1
Obese/EV
Lean/GLP-1
Lean/EV

Figure 34

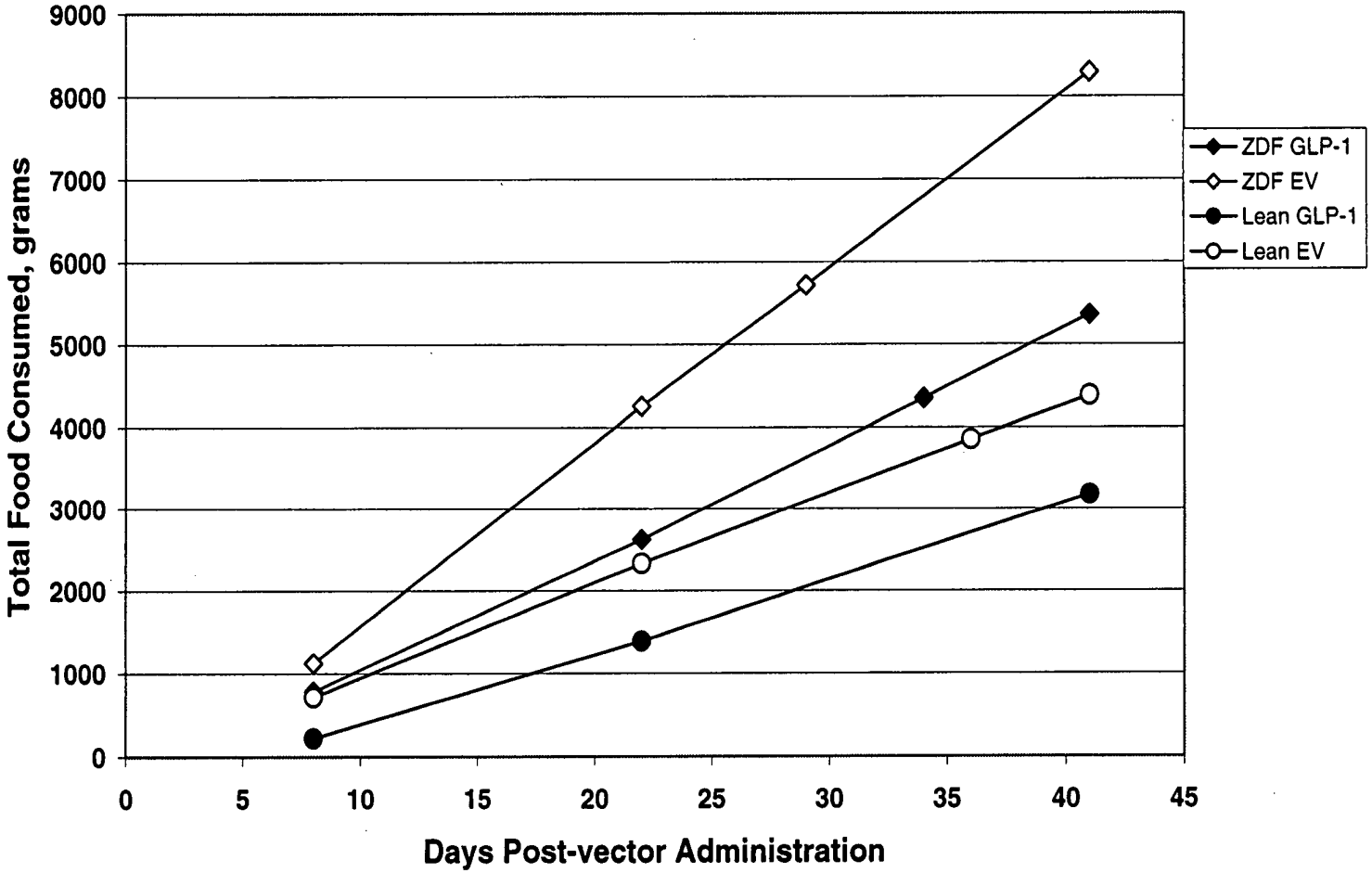

9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Total Food Consumed, grams
0
5
10
15
20
25
30
35
40
45
Days Post-vector Administration
ZDF GLP-1
ZDF EV
Lean GLP-1
Lean EV

Figure 35

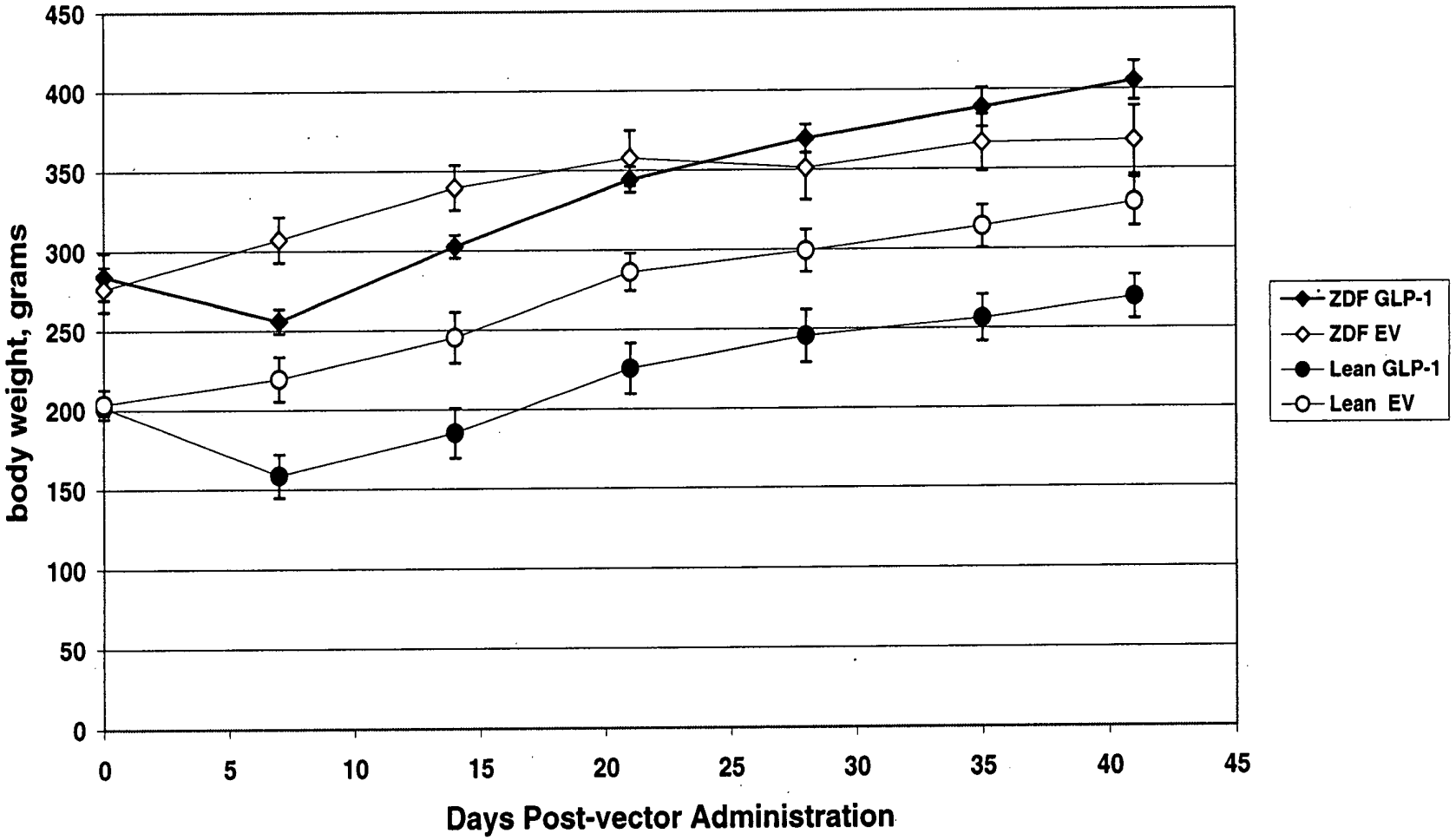
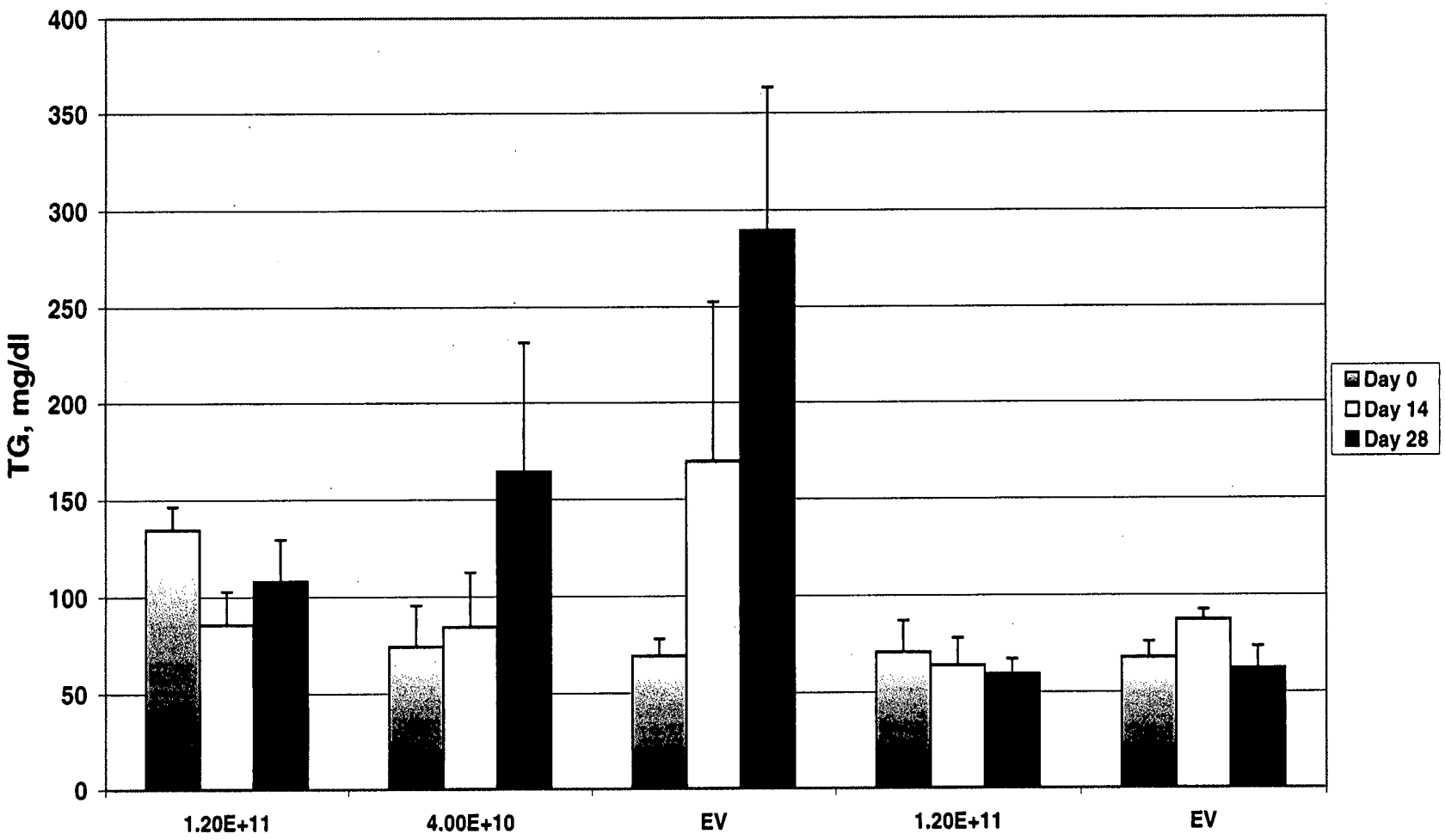

450
400
350
300
250
200
150
100
50
0
body weight, grams
0
5
10
15
20
25
30
35
40
45
Days Post-vector Administration
ZDF GLP-1
ZDF EV
Lean GLP-1
Lean EV

Figure 36


400
350
300
250
200
150
100
50
0
TG, mg/dl
1.20E+11
4.00E+10
EV
1.20E+11
EV
Day 0
Day 14
Day 28